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Antimicrobial Resistance of *E. Coli* in Commercial Broiler

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ABSTRACT

The antimicrobial resistance profile of *E. coli* isolates from cloacal and intestinal swabs of broilers in commercial poultry farms was studied. Susceptibility to nine antibiotic discs: amoxicillin (AML), clavulanic acid (CLA), enrofloxacin (ENR), gentamicin (GEN), oxytetracycline (OTC), trimethoprim (TMP), ciprofloxacin (CIP), doxycycline (DO) and tylosin (TYL) was tested on 50 isolates using the disc diffusion method (Kirby-Bauer). There was significant variance in the level of antibiotic efficiency, with the highest mean inhibition zone diameter recorded for Clavulanic Acid (20.21 mm) and the least for doxycycline (8.23 mm), suggesting high susceptibility and resistance. ANOVA indicated significant differences between antibiotics ($F = 12.52$, $p < 0.000001$), and Tukey's HSD post hoc test confirmed that Clavulanic Acid performed the best against other drugs. Multidrug resistance (MDR) (resistance to three or more antibiotics) was detected in 64.6% of isolates, with serious consequences for animal and public health. The researchers concluded that the high levels of resistance combined with the plasmid-mediated transfer of resistance make resistance treatment in the poultry sector a priority for responsible antibiotic use, farm-level interventions, and continued research on resistance mechanisms, environmental determinants of resistance, and alternatives to antibiotics.

INTRODUCTION

The poultry sector is a cornerstone of the global food system, supplying affordable protein and driving economic growth, particularly in Pakistan, where it contributes significantly to rural livelihoods, employment, and allied industries (Jassim & Shareef, 2023). However, this rapidly expanding sector faces increasing threats from bacterial infections, especially *Escherichia coli (E. coli)*, a commensal organism that can cause severe poultry diseases such as colibacillosis, septicemia, and enteritis, resulting in poor growth, mortality, and major financial losses (Lemlem et al., 2023). Beyond animal health, *E. coli* is a major foodborne pathogen, capable of causing gastrointestinal illness and life-threatening conditions like kidney failure in humans through contaminated or undercooked poultry products (Hasona, Helmy, & El Gamal, 2023). A pressing concern is the emergence of antimicrobial resistance (AMR). Misuse and overuse of antibiotics as prophylactics, growth promoters, and therapeutics in poultry farms accelerate the selection of resistant strains (Ibrahim et al., 2023). Resistant *E. coli* strains acquire and disseminate resistance genes via mobile genetic elements (plasmids, transposons), not only within poultry but also across the environment, contaminating soil, water, and crops. This facilitates transmission to humans through food, direct contact, or environmental exposure, undermining the effectiveness of antibiotics critical for both veterinary and human medicine (Ebrahem et al., 2024). Infections caused by multidrug-resistant (MDR) *E. coli* are harder to treat, leading to longer hospital stays, higher costs, and increased mortality (Lemlem et al., 2023). The public health implications are severe, as resistant strains threaten food safety and compromise treatment options for common infections. Antibiotics such as fluoroquinolones, cephalosporins, and tetracyclines used in both poultry and human medicine—have lost efficacy against resistant isolates, highlighting the risk of losing vital therapeutic tools (Jassim & Shareef, 2023). The persistence of resistant bacteria in farm manure and the environment further amplifies the spread and long-term impact of AMR. Given these risks, there is an urgent need for alternative strategies. Promising options include plant-based antimicrobials (e.g., extracts of *Mangifera indica*, *Glycyrrhiza glabra*, *Aloe vera*), probiotics that strengthen gut defenses, and bacteriophages, which target bacteria without disrupting beneficial microbiota (Bessalah et al., 2023; Ullah et al., 2023; Xu et al., 2022). While these approaches show potential to reduce antibiotic dependence and limit AMR development, more research is needed to optimize their effectiveness in poultry production systems (Alrasheid et al., 2023; Lemlem et al., 2023). While poultry remains vital to food security and economic development, the rise of MDR *E. coli* poses a dual threat to animal productivity and human health. Sustainable alternatives and stricter antibiotic stewardship are essential to safeguard both the poultry industry and public health.

MATERIAL AND METHODS

The study was designed to achieve the objectives outlined in the previous section through a series of systematic experimental investigations. Each stage of the methodology was designed to ensure accurate and reproducible results, to comprehensively analyze the antimicrobial resistance (AMR) profiles of *E. coli* isolated from poultry.

Study Period and Location

The one-year study combined field and laboratory experiments at Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi and the National Veterinary Laboratory, Islamabad. The university provided academic support and bacteriological facilities within its Department of Parasitology and Microbiology, while NVL offered advanced diagnostic and microbiological testing.

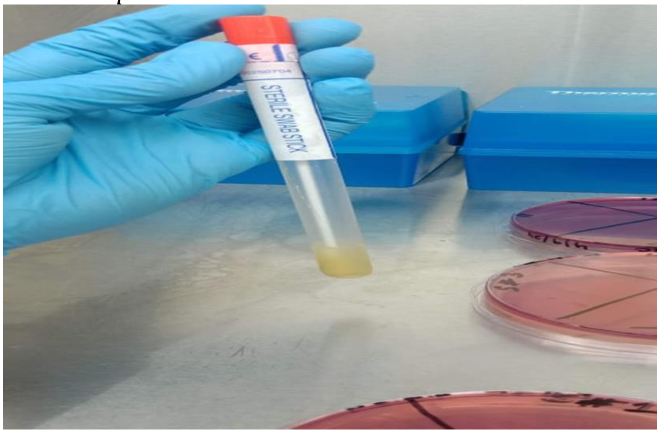
Material

E. coli was isolated from poultry intestinal and cloacal swabs using nutrient broth/agar and selective media such as MacConkey and EMB agar. Gram staining was performed to confirm bacterial identity, while Mueller-Hinton agar was used for antimicrobial susceptibility testing with antibiotic discs. These materials enabled reliable isolation, differentiation, and resistance profiling of E. coli strains.

Collection of Samples

Fifty intestinal and cloacal swabs were collected from commercial poultry farms in Khushab District, Pakistan, all with prior antibiotic use. Samples were preserved at 4 °C and processed for E. coli isolation, identification, and antimicrobial susceptibility testing. Of these, 43 (86%) tested positive, indicating a high prevalence suitable for AMR assessment.

Figure 1
Swab Sample



Isolation of *Escherichia coli*

Swab samples from live (cloacal) and dead (intestinal) birds were homogenized in phosphate-buffered saline and enriched in brain heart infusion broth, then incubated at 37 °C for 18–24 h. This enrichment promoted bacterial growth, facilitating successful isolation of *E. coli*. The enriched broth was streaked onto MacConkey agar, where *E. coli* appeared as pink lactose-fermenting colonies.

Figure 2
Streaking on MacConkey Agar

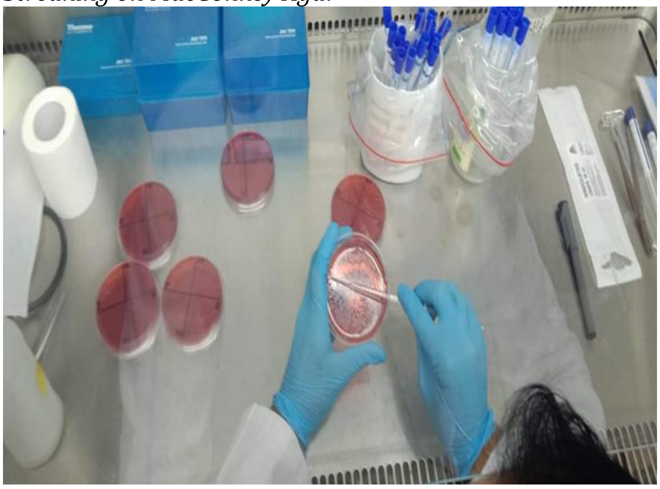


Figure 3
Appearance of Bacterial Colonies

Figure 4
Bacterial Growth on EMB Agar

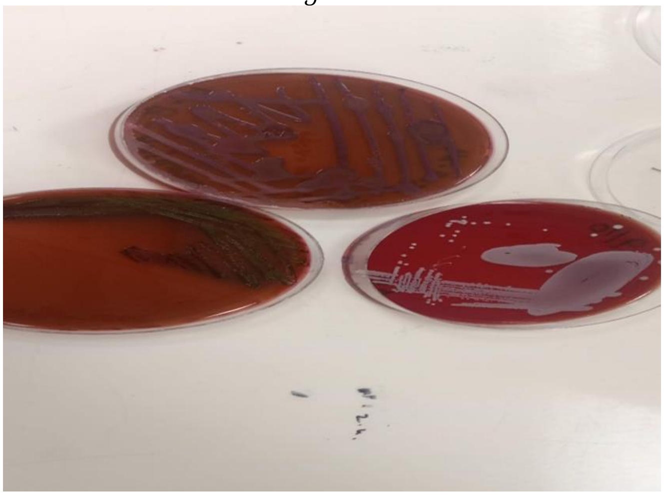
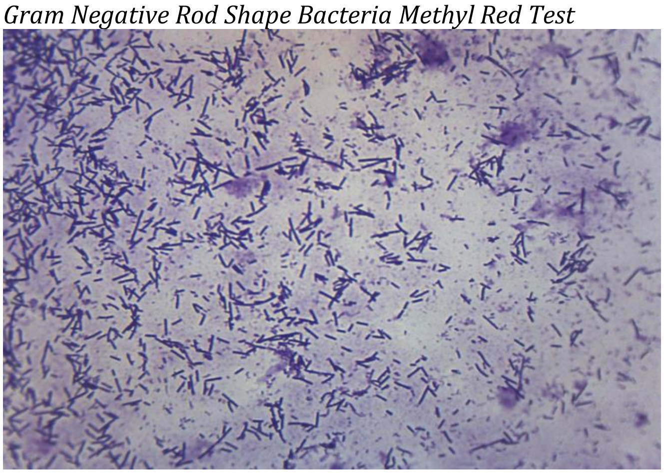


Figure 5
Gram Negative Rod Shape Bacteria Methyl Red Test



E. coli has the characteristics that it can ferment glucose by acid fermentation so methyl red test was performed to assess the bacteria's capability to ferment glucose.

Catalase Test

The presence of catalase can be confirmed by performing catalase test, which breaks down hydrogen peroxide into water and oxygen.

Figure 6
Test tubes for Methyl Red

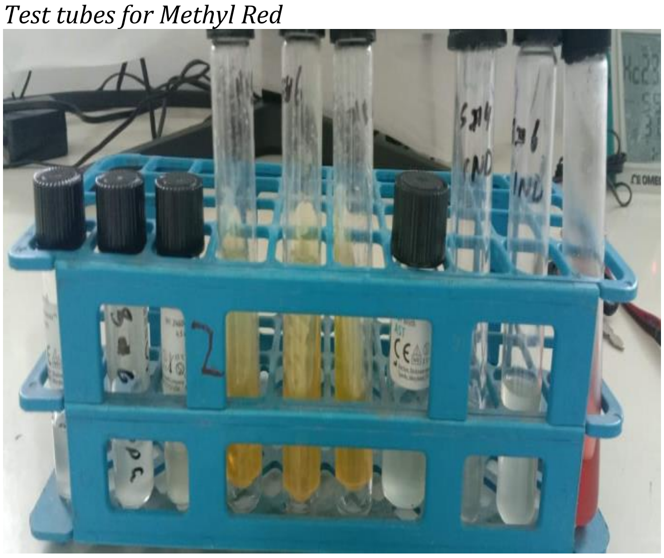
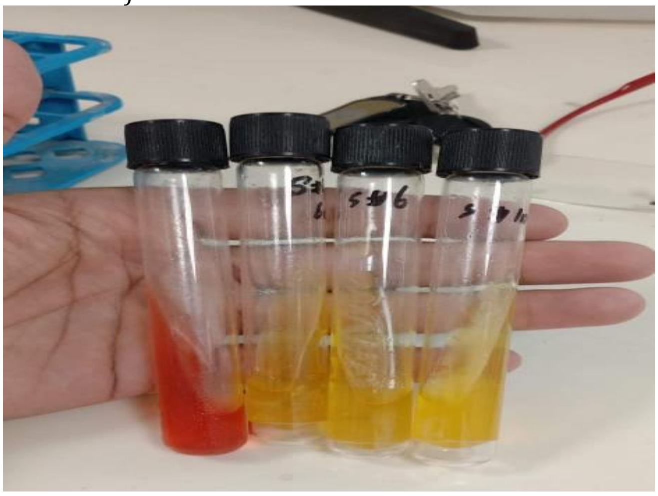


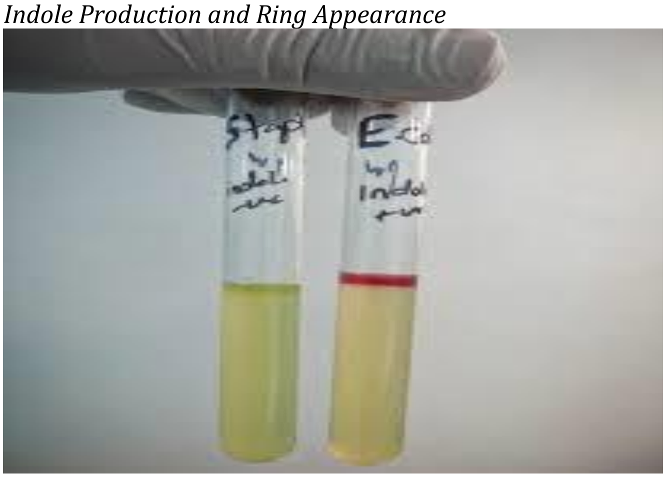
Figure 7
Test Tubes for Catalase Test



Indole Test

Tryptophan which is an amino acid, can be decomposed by an enzyme which is present its *E. coli.* The enzyme tryptophanase, produced by *E. coli*, catalyzes this reaction.

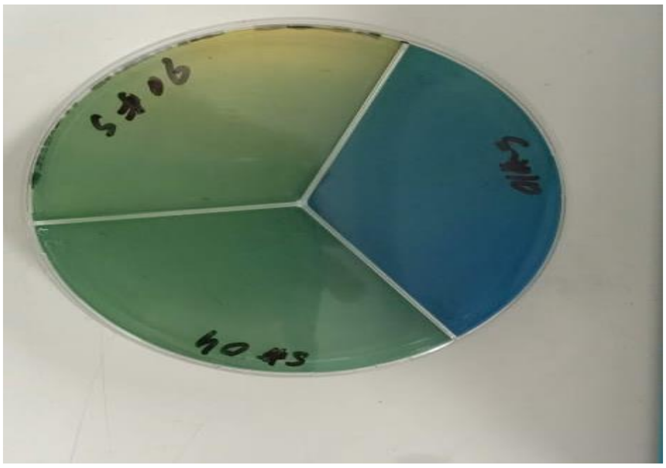
Figure 8
Indole Production and Ring Appearance



Citrate Utilization Test

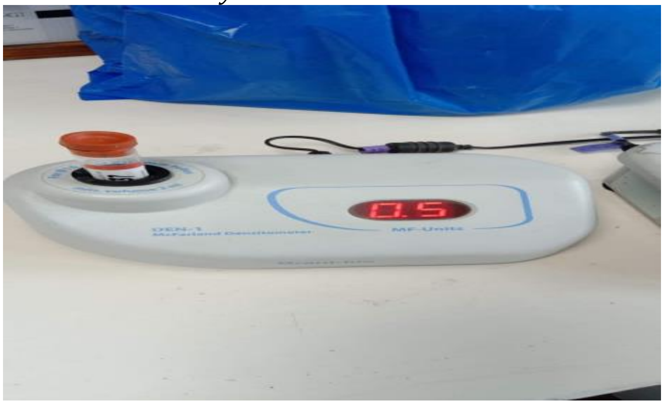
It evaluates the capacity of bacteria to utilize citrate as the only carbon source. *E. coli* cannot utilize citrate for growth, so it would not change color in the medium containing citrate.

Figure 9
Citrate Utilization Test



Antibiotic Susceptibility Test

Figure 10
McFarland Turbidity Meter



E. coli samples were collected from broiler farms, and their antibiotic susceptibility was assessed using the Kirby–Bauer disc diffusion technique. Bacterial suspensions were prepared at a concentration of 0.5 McFarland and swabbed on Muller Hilton agar plates using the quadrant streak method.

Figure 11
Placement with Antibiotic Discs on MH Agar

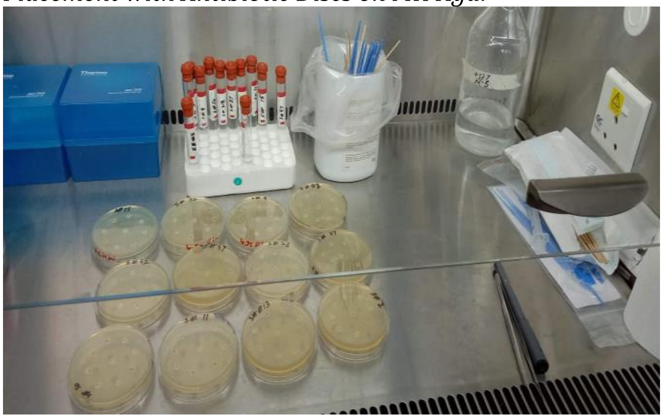


Figure 12
Antibiotic Sensitivity Result

Statistical Analysis

The data obtained from the antibiotic susceptibility testing were analyzed using SPSS software to determine the statistical significance of the results. The analysis was carried out at a significance level of P<0.05 to identify patterns in the AMR profiles across different samples. This statistical approach helped to evaluate the relationships between various factors, including antibiotic usage, farm management practices, and resistance patterns, providing insights into the factors driving AMR in poultry.

RESULTS

Isolation and Identification of *Escherichia coli*

Out of 50 cloacal and intestinal swabs from broiler farms in Khushab, 43 (86%) yielded *E. coli*. Isolates produced pink colonies on MacConkey agar, metallic green sheen on EMB, and were confirmed as gram-negative rods by staining and biochemical tests (Indole and MR positive, Citrate negative).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of 43 *E. coli* isolates was assessed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, following CLSI M100 guidelines. Nine antibiotics were tested, including clavulanic acid (CAL 40µg), enrofloxacin (ENR 5µg), gentamicin (CN 10µg), oxytetracycline (OT 30µg), trimethoprim (5µg), amoxicillin (AX 25µg), ciprofloxacin (CIP 5µg), doxycycline (DXT 30µg) and tylosin (TY 30µg). This approach evaluated resistance patterns relevant to both veterinary and human medicine.

Figure 13
Antimicrobial Susceptibility Test

Figure 14
Antimicrobial susceptibility of 43 E. coli *isolates was tested using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar under CLSI M100 guidelines. Nine commonly used veterinary and human antibiotics were evaluated to determine resistance patterns*

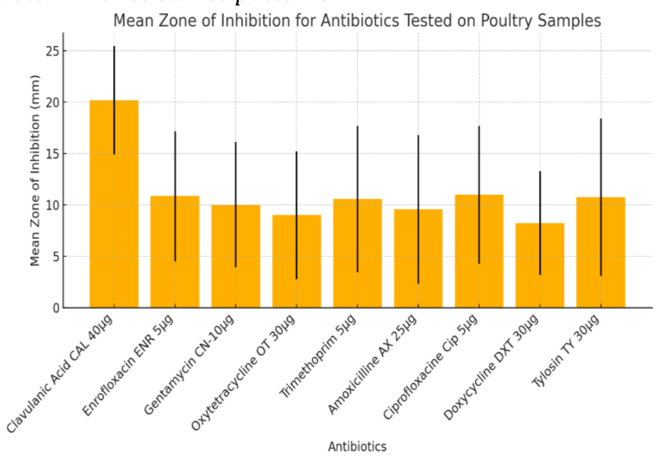


Table 1

Descriptive Statistics for Antibiotic Resistance Data

Antibiotic	n	Mean	SD	Median	Min	Max	Range	SE
Clavulanic Acid CAL 40µg	43	20.21	5.32	20	11	30	19	0.81
Enrofloxacin ENR 5µg	43	10.86	6.42	11	0	22	22	0.98
Gentamycin CN-10µg	43	10.02	6.17	11	0	19	19	0.94
Oxytetracycline OT 30µg	43	9.02	6.29	11	0	19	19	0.96
Trimethoprim 5µg	43	10.56	7.18	11	0	26	26	1.1
Amoxicilline AX 25µg	43	9.58	7.33	10	0	28	28	1.12
Ciprofloxacine Cip 5µg	43	11.0	6.77	11	0	30	30	1.03
Doxycycline DXT 30µg	43	8.23	5.09	9	0	16	16	0.78
Tylosin TY 30µg	43	10.77	7.75	11	0	30	30	1.18

Figure 15

The violin plot shows inhibition zone distributions for tested antibiotics, with medians and interquartile ranges embedded. Clavulanic acid and doxycycline displayed higher activity, while amoxicillin and trimethoprim showed weaker effects.

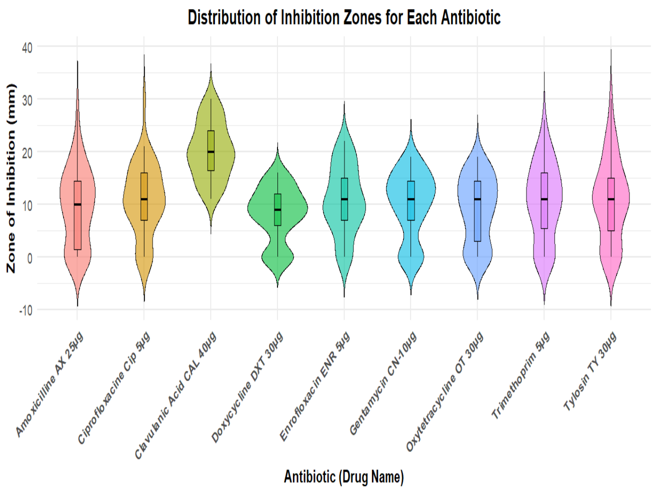


Figure 16

The donut chart shows antibiotic effectiveness by mean inhibition zones, highest for clavulanic acid (20.2%), ciprofloxacin (11%), and enrofloxacin (10.8%). Doxycycline, oxytetracycline, and amoxicillin was less effective.

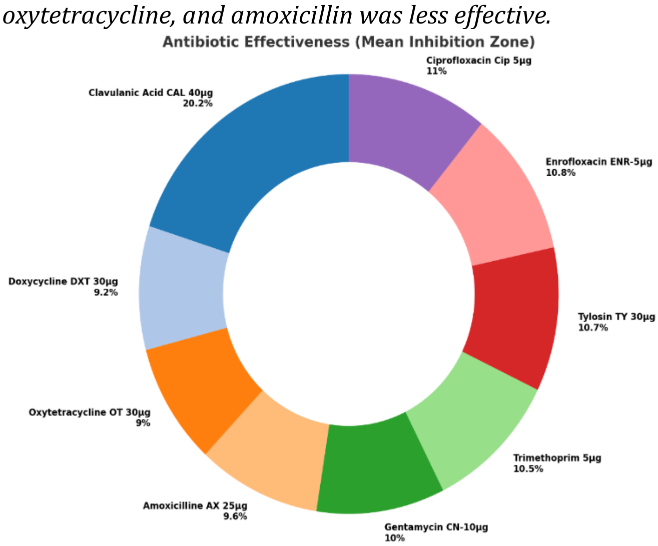


Figure 17

The box plot compares inhibition zone diameters, showing clavulanic acid with the highest consistent activity, while amoxicillin and doxycycline were less effective and ciprofloxacin showed variability.

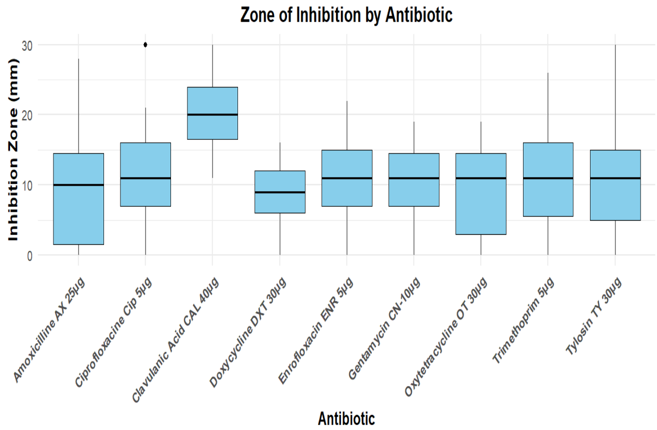
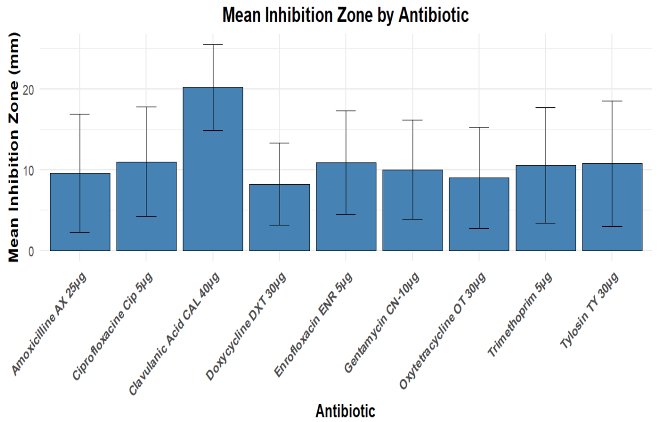


Figure 18

The bar chart shows Clavulanic Acid with the highest mean inhibition (>20 mm), while ciprofloxacin, enrofloxacin, trimethoprim, and doxycycline displayed moderate activity with varying variability.



Statistical Comparison of Antibiotic Efficacy (ANOVA)

A one-way ANOVA was performed to assess differences in inhibition zones among antibiotics, showing a highly significant effect ($F\ (8, 378) = 12.52$; $p < 0.000001$). This indicates the differences in antimicrobial efficacy are statistically meaningful, not random. It is represented in table 2.

Table 2

One-Way ANOVA Summary for Zone of Inhibition by Antibiotic

Source	Df	Sum of Squares	Mean Square	F Value	Pr(>F)	Significance
Antibiotic	8	4276	534.5	12.52	5.77e-16	***
Residuals	378	16137	42.7			

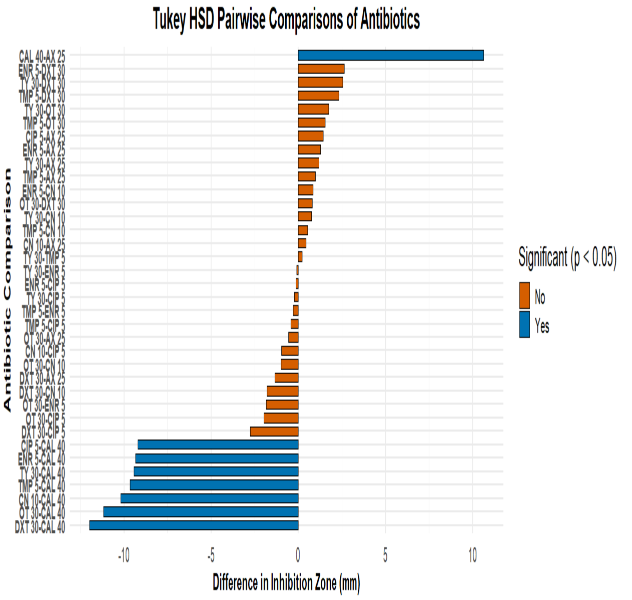
Post HOC Analysis (Tukey HSD Test)

A Tukey HSD post hoc test identified significant pairwise differences in antibiotic efficacy. Clavulanic Acid showed markedly higher inhibition zones than amoxicillin, doxycycline, and oxytetracycline, confirming its superior performance. Ciprofloxacin also differed significantly from trimethoprim and tylosin, indicating moderate quinolone efficacy. The full list of statistically significant pairwise comparisons is detailed in Table 3. These comparisons are visually summarized in Figure 19.

Table 3
Tukey HSD Pairwise Comparisons

Comparison	diff	Lw	up	p adj
Ciprofloxacine Cip 5µg – Amoxicilline AX 25µg	1.418605	-2.977600	5.814809	0.985130
Clavulanic Acid CAL 40µg – Amoxicilline AX 25µg	10.627907	6.231702	15.024112	0.000000
Doxycycline DXT 30µg – Amoxicilline AX 25µg	-1.348837	-5.745042	3.047367	0.989288
Enrofloxacin ENR 5µg – Amoxicilline AX 25µg	1.279070	-3.117135	5.675274	0.992472
Gentamycin CN-10µg – Amoxicilline AX 25µg	0.441860	-3.954344	4.838065	0.999997
Oxytetracycline OT 30µg – Amoxicilline AX 25µg	-0.558140	-4.954344	3.838065	0.999983
Trimethoprim 5µg – Amoxicilline AX 25µg	0.976744	-3.419460	5.372949	0.998857
Tylosin TY 30µg – Amoxicilline AX 25µg	1.186047	-3.210158	5.582251	0.995493
Clavulanic Acid CAL 40µg – Ciprofloxacine Cip 5µg	9.209302	4.813098	13.605507	0.000000
Doxycycline DXT 30µg – Ciprofloxacine Cip 5µg	-2.767442	-7.163646	1.628763	0.569557
Enrofloxacin ENR 5µg – Ciprofloxacine Cip 5µg	-0.139535	-4.535739	4.256670	1.000000
Gentamycin CN-10µg – Ciprofloxacine Cip 5µg	-0.976744	-5.372949	3.419460	0.998857
Oxytetracycline OT 30µg – Ciprofloxacine Cip 5µg	-1.976744	-6.372949	2.419460	0.896379
Trimethoprim 5µg – Ciprofloxacine Cip 5µg	-0.441860	-4.838065	3.954344	0.999997
Tylosin TY 30µg – Ciprofloxacine Cip 5µg	-0.232558	-4.628763	4.163646	1.000000
Doxycycline DXT 30µg – Clavulanic Acid CAL 40µg	-11.976744	-16.372949	-7.580540	0.000000
Enrofloxacin ENR 5µg – Clavulanic Acid CAL 40µg	-9.348837	-13.745042	-4.952633	0.000000
Gentamycin CN-10µg – Clavulanic Acid CAL 40µg	-10.186047	-14.582251	-5.789842	0.000000
Oxytetracycline OT 30µg – Clavulanic Acid CAL 40µg	-11.186047	-15.582251	-6.789842	0.000000
Trimethoprim 5µg – Clavulanic Acid CAL 40µg	-9.651163	-14.047367	-5.254958	0.000000
Tylosin TY 30µg – Clavulanic Acid CAL 40µg	-9.441860	-13.838065	-5.045656	0.000000
Enrofloxacin ENR 5µg – Doxycycline DXT 30µg	2.627907	-1.768298	-7.024112	0.638354
Gentamycin CN-10µg – Doxycycline DXT 30µg	1.790698	-2.605507	6.186902	0.939189
Oxytetracycline OT 30µg – Doxycycline DXT 30µg	0.790698	-3.605507	5.186902	0.999759
Trimethoprim 5µg – Doxycycline DXT 30µg	2.325581	-2.070623	6.721786	0.776042
Tylosin TY 30µg – Doxycycline DXT 30µg	2.534884	-1.861321	6.931088	0.682952
Gentamycin CN-10µg – Enrofloxacin ENR 5µg	-0.837209	-5.233414	3.558995	0.999631
Oxytetracycline OT 30µg – Enrofloxacin ENR 5µg	-1.837209	-6.233414	2.558995	0.929879
Trimethoprim 5µg – Enrofloxacin ENR 5µg	-0.302326	-4.698530	4.093879	0.999999
Tylosin TY 30µg – Enrofloxacin ENR 5µg	-0.093023	-4.489228	4.303181	1.000000
Oxytetracycline OT 30µg – Gentamycin CN-10µg	-1.000000	-5.396205	3.396205	0.998645
Trimethoprim 5µg – Gentamycin CN-10µg	0.534884	-3.861321	4.931088	0.999988
Tylosin TY 30µg – Gentamycin CN-10µg	0.744186	-3.652019	5.140391	0.999847
Trimethoprim 5µg – Oxytetracycline OT 30µg	1.534884	-2.861321	5.931088	0.975549
Tylosin TY 30µg – Oxytetracycline OT 30µg	1.744186	-2.652019	6.140391	0.947612
Tylosin TY 30µg – Trimethoprim 5µg	0.209302	-4.186902	4.605507	1.000000

Figure 19
The Tukey HSD comparison shows mean differences in inhibition zones, with blue bars indicating significant ($p < 0.05$) results and orange bars non-significant. Clavulanic Acid consistently showed significantly higher inhibition than all other antibiotics, underscoring its superior efficacy.



DISCUSSION

The study revealed that *E. coli* isolates from broilers exhibited diverse AMR profiles, with Clavulanic Acid (CAL 40µg) showing the highest mean inhibition zones, confirming its strong potential against MDR *E. coli* (Aberkane et al., 2023). This supports the study objective of assessing AMR trends in poultry, highlighting that resistance is highest to commonly used antibiotics such as doxycycline and amoxicillin, and lower to less frequently used drugs (Urumova et al., 2024). These findings fill a gap in local AMR data and strengthen antimicrobial stewardship strategies in the poultry sector. Comparisons with other research show consistency, as high resistance to tetracyclines and penicillins has also been reported elsewhere (Ibrahim et al., 2023). However, unlike earlier studies where fluoroquinolones or aminoglycosides were most effective (Mesa-Varona et al., 2021; Truswell et al., 2023), this study identifies Clavulanic Acid as superior. The novelty lies in the broad antibiotic panel tested, supported by robust ANOVA and Tukey HSD analyses, which provide a detailed, region-specific understanding of AMR. Limitations include a small sample size, cross-sectional design, reliance on phenotypic methods without molecular confirmation, and sampling limited to Khushab

District, restricting generalizability (Lin et al., 2025). Future research should expand sampling, incorporate molecular diagnostics, and evaluate alternative treatments such as probiotics and bacteriophages. Practically, the findings stress the need for stronger antimicrobial stewardship, routine susceptibility testing before treatment, and adoption of sustainable alternatives. Such measures will enhance disease control and help mitigate AMR risks in Pakistan's poultry industry (Mudenda et al., 2022; Laopiem et al., 2025).

CONCLUSION

This study revealed significant variation in antimicrobial resistance (AMR) among *E. coli* isolates from commercial broilers, with most showing robust resistance patterns. Clavulanic Acid (CAL 40µg) emerged as the most effective antibiotic, while commonly used drugs such as doxycycline, amoxicillin, and oxytetracycline showed limited efficacy. These findings confirm the widespread presence of multidrug resistance (MDR) in poultry-associated *E. coli* and provide valuable insight into resistance trends in Pakistan's poultry sector. The results underscore the need for rational antibiotic selection, stricter antimicrobial use in broiler production, and improved stewardship practices to mitigate the risks of MDR.

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