Journal of Animal & Plant Sciences, 35(6): 2025, Page: 1585-1598
ISSN (print): 1018-7081; ISSN (online): 2309-8694

Original Article

THE ASSESSMENT OF ANTIBIOTICS USAGE AND DISEASE TRENDS IN COMMERCIAL BROILER FARMS OF POTOHAR REGION IN PAKISTAN: IMPLICATIONS FOR FOOD SAFETY

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ABSTRACT

Pakistan's agriculture sector contributing 14.63% to the GDP in 2024 is majorly supported by the poultry industry, with a population of more than one billion broiler chickens. This research was conducted, in which data was collected from March 2022 to December 2023 through a structured questionnaire and analyzed by using univariate and multivariate methods, including multiple correspondence analyses (MCA) and cluster dendrogram. This study identified enteritis (38.46%), bronchitis (19.23%) and Newcastle disease (15.38%) as the most prevalent diseases. The antibiotic colistin, which is considered as a last resort antibiotic in poultry production, was found most frequently used (76.92%) followed by quinolones (65.38%), amoxicillin (53.84%), macrolides (34.61%), and aminoglycosides (30.76%). The antibiotics sulfonamides (15.38%), tetracyclines (11.53%) and nitrofurans (3.84%) were found to be used less commonly in farm management practices. These findings highlight an overuse of colistin and other antibiotics, mostly in combination, posing a significant threat to public health for the possible emergence of antimicrobial resistance. Our findings elucidate the vitality of educational interventions and farming-oriented training in the improvement of management strategies and sustainable farming practices. By addressing the extra-label uses of antibiotics in broiler farms, Pakistan can take major steps towards alleviating the global threat of antibiotic resistance while ensuring food safety and public health.

Key Words: Antibiotics Resistance, Diseases, Broiler Farm, Colistin, Public Health.

Published first online September 22, 2025 Published final November 30, 2025

INTRODUCTION

Pakistan's agriculture sector has changed rapidly over the last three decades, evolving from conventional farming approaches to modern farming systems, including intensive livestock and poultry production. Increasing intensive animal production has led to the excessive use of antibiotics for growth promotion, prevention and treatment of various diseases. Pakistan is an agricultural country and its livestock production contributed 14.97 % to the national GDP in the financial year 2024-25 (Anonymous 2025). The poultry industry is an important part of the livestock industry, with a bird population of 17.44 million parents, 83.90 million layers and approximately 2.06 billion broilers. Poultry accounts for 43.3% of total meat consumption in Pakistan, which is approximately 2.58 million tonnes of meat annually (Anonymous 2025). The increased use of antibiotics in poultry production, therefore, has a significant impact on the development of antibiotic resistance in poultry bacteria and its possible spread to humans and the environment, be it through direct contact with animals or animal waste or through the consumption of contaminated food (Vieira *et al.,* 2011; Krishnasamy *et al.,* 2015; Zhang *et al.,* 2015).

Previous research has mainly focused on the occurrence of antibiotic-resistant bacteria and antibiotic-resistance patterns in poultry production in Pakistan (Rahman and Mohsin, 2019). However, there is limited knowledge about antibiotic use practices in poultry farms. Antibiotic practices in broiler production farms may be related to the farmer's educational status, level of farming training and background knowledge about the purpose of antibiotics and antibiotic resistance. Farmers knowledge improvement, understanding of antibiotic resistance and justified antibiotic use can help and establish more responsible antibiotic use, leading to reduced development and spread of antibiotic resistance (Sirdar *et al.,* 2012; Om and McLaws*,* 2016). There are multiple research studies conducted that explored the antibiotic use practices of poultry farmers and the factors contributing to antibiotics misuse in Pakistan (Habiba *et al.,* 2023;Rasheed *et al.,* 2025).

Extra-label use of antibiotics in food animal production has become a major key contributor in the global spread of antimicrobial resistance both in animals and humans, particularly in low and middle-income countries (LMICs) (Nayiga *et al.,* 2020). Antimicrobial resistance has been continuously increasing over the last decades; it is currently estimated to be responsible for over 7 million deaths annually, and it is estimated to cause approximately 10 million deaths by the year 2050 (Naghavi *et al.,* 2024). Excessive antibiotic usage in broiler farms remains a critical issue worldwide, evident in countries like China, Brazil and India where prophylactic and growth-promoting antibiotics are widely used (Van Boeckel *et al.,* 2019). Antibiotics are used in Pakistan's poultry sector for growth promotion, disease prevention and treatment in huge amounts without oversight by veterinary professionals (Umair *et al.,* 2021). This inappropriate use of antibiotics contributes to the development and transmission of antibiotic resistance genes, posing a serious public health concern. Inappropriate policies and regulations for antibiotic usage in LMICs have led to drug-resistant infections, leading to the overuse of antibiotics in animals and humans (Otaigbe *et al.,* 2023). Additionally, resistance determinants, i.e. both resistant bacteria and resistance genes, can be spread from infected animals or humans into the environment (Van Boeckel *et al.,* 2015). Direct connection with animals or the environment and consumption of contaminated food are major paths for zoonotic transmission of resistance (McEwen and Collignon, 2018).

The lack of valid antibiotic usage and antibiotic resistance data from behavioral and social perspectives is essential to get attitudinal changes on the misuse of antibiotics to eliminate the antimicrobial resistance threat. Hence, improvement of farmer's and people's awareness and training and knowledge of the misuse of antibiotics will majorly contribute to the reduction of antibiotic usage and consumption as well as antimicrobial resistance in society (Al-Mustapha *et al.,* 2020). To the best of our knowledge, this is the first comprehensive field-based study of antimicrobial usage and technical farm practices of commercial broiler farms in Potohar region, Pakistan. It is based on field survey data and information obtained directly from farm owners and managers throughout the weeks of the flock production cycle. Therefore, this present study aimed to assess farming practices, disease prevalence and medication, including antibiotic usage patterns in broiler flocks and their effect on flock performance by using univariate and multivariate statistical analyses.

Study Design: A comprehensive study was carried out on commercial broiler farming practices in the Potohar region of Pakistan between March 2022 to December 2023. A cross-sectional survey was conducted in the districts Rawalpindi, Attock, Chakwal, Khushab and Haripur which were selected for this study (Figure 1). The northern Punjab region has a high density of poultry farms and reflects a diverse combination of commercial and rural farming systems. A total of 26 conventional and modern farms with a cumulative bird population of 0.618 million (farm sizes range 3000-30000 birds in each shed) were selected among these districts to be included in the study. The farms provided informed consent and agreed to share accurate production and health records. We excluded those farms that didn't provide accurate farm data. The information was collected from farm owners and supervisors by conducting detailed interviews.

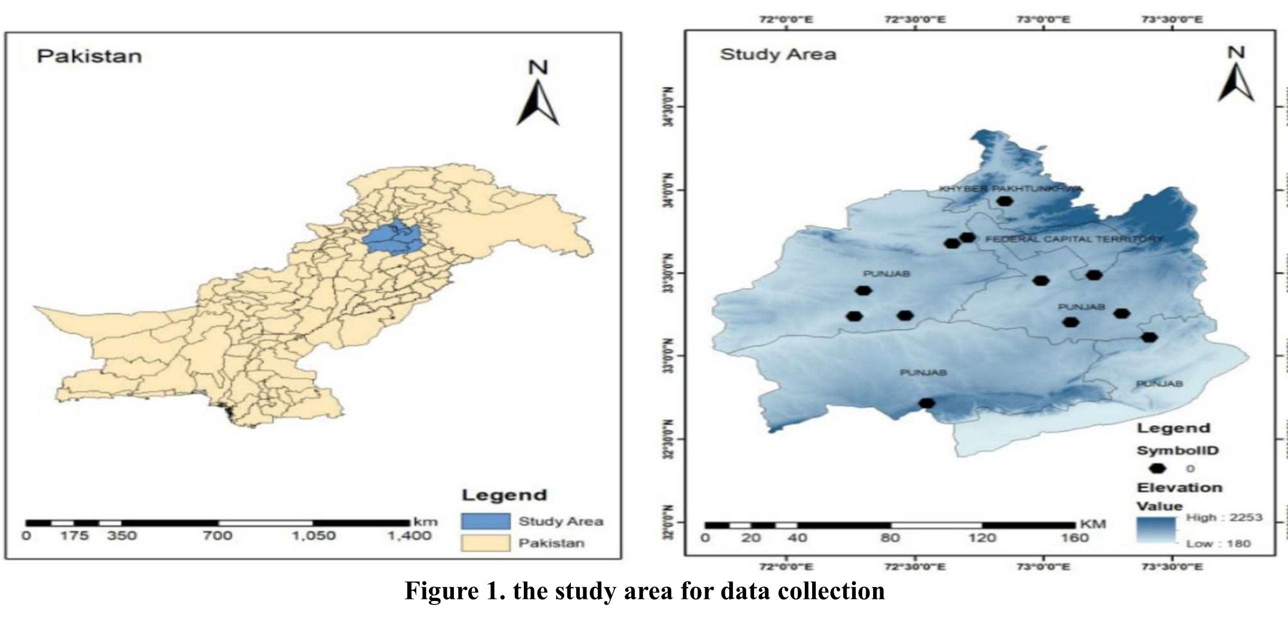

Figure 1. the study area for data collection

Data Collection: A questionnaire was developed to collect the data following a similar format of the questionnaire used in previous studies from China (Al-Mustapha *et al.,* 2020). The farm data was collected using a pre-tested survey using researchers administered questionnaire/data collection tool (DCT). The research questionnaire consisted of five main sections: 1) Farm characteristics and farm management protocols; 2) Sociodemographic characteristics of farmers; 3) Farmer's strategies for disease prevention and control; 4) Training of farmers and their technical staff on the use of antibiotics and their knowledge on antibiotic resistance; 5) Mortality, weight gain and feed conversion ratio of each farm. The questionnaires were completed by the researcher during the first visit to the farms, and each interview lasted approximately one hour. Each farm was then visited once per week (five visits in total), to record the average weight, mortality, medications and feed conversion ratio of the flock during the first to fifth weeks of production. The study was approved by the Ethical Research Committee of Quaid-i-Azam University, Islamabad, Pakistan No.#BEC-FBS-QAU2023-532.

Data Management and Analysis: Data sheets, prepared using Microsoft Excel 2016 and RStudio IDE (version 2024.09.1, Build 394) were used for further univariate and multivariate analysis. Univariate analysis was performed on weight gain, mortality and antibiotic usage, whereas multivariate analysis included multiple correspondence analysis (MCA) on management-related categorical variables and cluster analysis on weekly flock performance data. To ensure standardized data collection, we used a structured questionnaire across all farms, with consistent variables defined. Categorical variables used in MCA were selected and defined to minimize bias and ensure comparability across responses.

The results of the categorical variables in Table 1 were used as input for the multiple correspondence analysis , to examine clusters of similarity among the farms included in the study. MCA was performed using the R package FactoMineR (Husson *et al.,* 2011).

Table 1. Data on the variables listed in the table were collected for each flock.

Variable name	Variable explanation
Week	Flock production week
Flock average weight	The average weight of the flocks in each week of production by doing the weight of 1% birds in the flocks
Mortality	Number of dead broilers in each production week
Farm owner education	Farmers education level
Farm owner training	Farmer training in poultry production and health management
Farm supervisor education	Supervisor education level
Farm supervisor training	Supervisor professional training in poultry production
Antibiotic resistance knowledge	Knowledge about the use of antibiotics and resistance
Antibiotic sensitivity benefits knowledge	Benefits of laboratory testing for choosing the right antibiotic
Do antibiotics increase weight gain?	The farmer's knowledge of the antibiotic effect on weight gain
Biosecurity	Are farmers following biosecurity protocols?

The weight gain was determined by the initial average weight of the birds and the final weight at the end of each production week. Mortality, medication including antibiotics and disease recorded weekly for the 26 flocks were also subjected to cluster analysis, to identify clusters of similarity among the flocks across a production cycle. Cluster analysis was performed with PC-Ord5 using the Jaccard grouping and Ward's linkage method (McCune and Mefford, 2011).

RESULTS

The flock and farm characteristics of the surveyed farms (N=26) revealed crucial operational and demographic insights. All the farmers were male (100%), with the majority (61.54%) having completed education equal to matriculation. Table 2 further demonstrates the execution of biosecurity practices (80.77%) and full flock vaccination (84.61%) across most farms. Additionally, the table also provides statistics on farmer experience, antimicrobial resistance awareness knowledge, and the prevalence of common infections or diseases on farms such as bronchitis, enteritis and new castle disease.

MCA-based correlation pattern of farm practices: Our multiple corresponding analysis reflected the indispensable relationship between educational levels with farming practices across different dimensions.

In Figure 2A&B, in the horizontal axis (Dim 1) individuals with a primary education level (“primary”) have a coordinate of 1.377 and those uneducated (“uneducated”) a coordinate of 2.005 - both particularly linked with less suitable farming practices, such as lack of biosecurity measures (“biosecurity_0”) and partial flock vaccination (“partially_vaccinated”). On the other hand, individuals with high education backgrounds like graduates (coordinate -0.754 on Dim 1), and master's degree holders (coordinate 0.284 on Dim 1) are aligned with better farming practices, such as full flock vaccination ("fully_vaccinated") and implementation of biosecurity measures ("biosecurity_1"). The second dimension (Dim 2; vertical axis) confirms these clusters observed in Dim 1, and further explains differences in practices related to training and knowledge of farm owners and supervisors. Farm owners without training ("farm_owner_training_0") have a coordinate of -0.510 and those who got training ("farm_owner_training_1") a coordinate of 0.595.The same pattern was observed with farm supervisors education, with a coordinate of -0.154 on Dim 2 for uneducated supervisors ("farm_supervisor_education_0") and 0.514 for educated ones ("farm_supervisor_education_1"). Coincidently, a higher Dim 2 coordinate (0.154) was found for farmers who have better knowledge about antibiotic resistance, compared to those with no knowledge about antibiotic resistance (coordinate -0.096). These findings elucidate the vitality of educational interventions and farming-oriented training in the improvement of management strategies and sustainable farming practices.

Cluster dendrogram based on farm practices: The findings of the Cluster analysis show the proximity of the 26 flocks, at each of the five consecutive visit weeks, according to the distribution of the variables (weekly weight gain, mortality pattern) registered weekly for each flock (Figure 3). There is a clear separation between observations documented in the 1st week, 2nd and 3rd weeks, and 4th and 5th weeks. The flocks sampled during week 1 formed a unique cluster, suggesting uniformity in managemental practices, including consistent supplement strategies and prophylactic antibiotic use across the farms. In contrast, week 5 flocks showed more dispersed clustering, indicating divergent late-cycle health outcomes and intervention strategies among farms. The clustering of weeks 2 and 3, and weeks 4 and 5 observations indicates that there is less weekly variation observed in the measured variables among those weeks, than compared to prior and/or posterior weeks.

Table 2. This table shows a summary of responses to categorical variables during the interviews with the 26 participating farms is summarized below.

Farm and Flock characteristics (N=26)	Yes n (%)	NO n (%)
Gender of Farm Owner		
Male	26(100%)	-
Female	0(0%)	
Farm Owner Education		
Masters	2(7.69%)	
Graduation	4(15.38%)	
Matric	16(61.54%)	-
Primary	2(7.69%)	
Uneducated	2(7.69%)	
Farm Owner Training	12(46.15%)	14(53.85%)
Farm Supervisor Education		
Literate (Matric/Diploma)	6(23.07%)	-
Illiterate	20(76.93%)	-
Farm Supervisor Training	6(23.07%)	20(76.93%)
Antibiotic Resistance Knowledge	10(38.46%)	16(61.54%)
Farming Experience Owner Years		
>15	11(42.3%)	-
<15	15(57.7%)	
Flock Vaccination		
Fully Vaccinated	22 (84.61%)	-
Partially Vaccinated	4(15.39%)	
Antibiotic Culture Sensitivity Benefit Knowledge	16(61.54%)	10(38.46%)
Does Antibiotic Increase Weight Gain	12(46.15%)	14(53.85%)
Biosecurity	21(80.77%)	5(19.23%)
Number of Antibiotics Used in Flocks		
Low Usage (1-2)	8(30.76%)	-
High Usage (3-7)	18(69.24%)	
Occurrence of Diseases		
New Castle Disease	4(15.38%)	
Enteritis	10(38.46%)	
Bronchitis	5(19.23%)	-
Multiple Diseases	4(15.38%)	
No Disease	3(11.54%)	

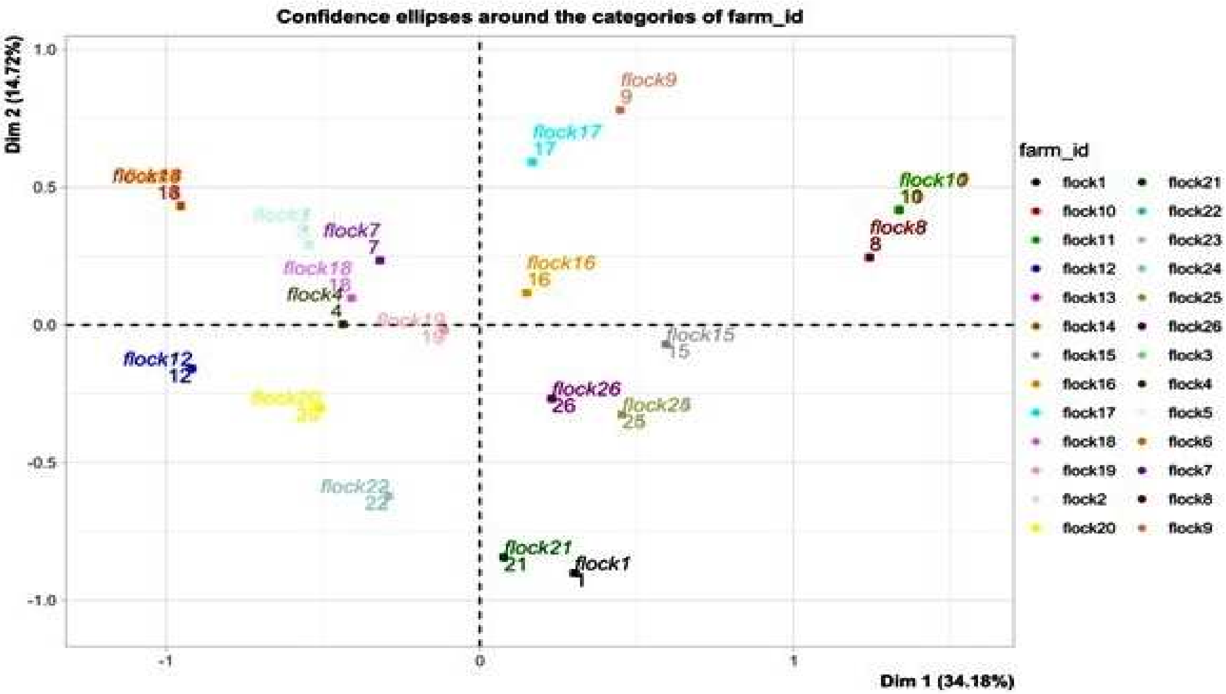

Figure 2A. MCA analysis of various broiler farms

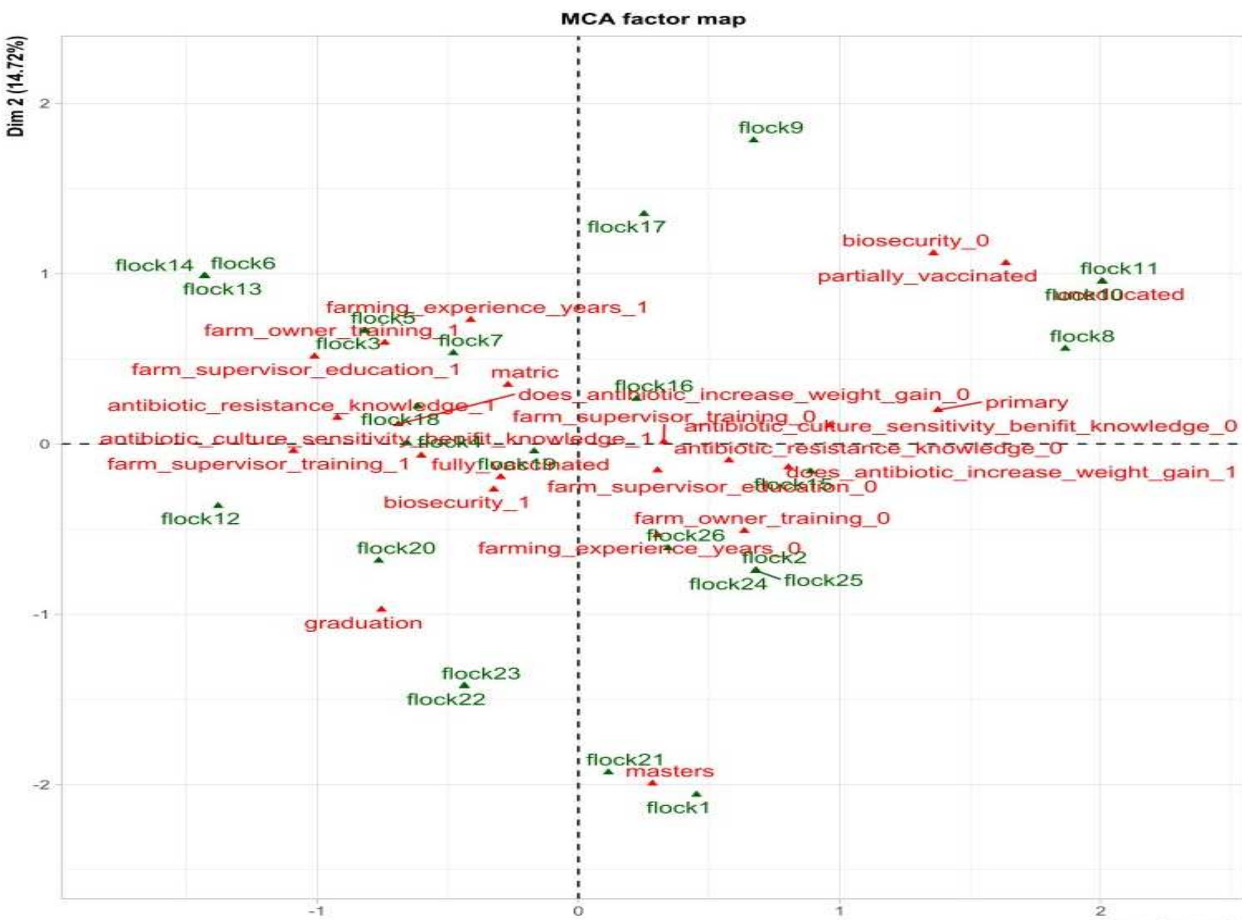

Figure 2B. MCA analysis of based on farm questionnaire data

Flocks with similar production patterns tended to cluster together. While the position of most flocks in the clusters varied along the 5-week cycle, some groups of flocks consistently clustered together, and in accordance with their positions observed in the MCA (Figure 2). Flocks F8 and F9 clustered together in all weeks, and flocks F16 and F17 in weeks 1 to 4. These four flocks also grouped together in the MCA, and represented flocks with low biosecurity, poor vaccination status and uneducated farmers. Similarly, flocks F24 and F25 also clustered together through weeks 1 to 5 and were jointly located in the same quadrant in the MCA, overlapping with the categories of low level of training among farm owners and supervisors, and low level of farming experience. Contrarily, flocks F6 and F5 and flocks F14 and F18 clustered together in weeks 2 to 5 and were also located in the same quadrant in the MCA biplot, coinciding with a high level of training and experience of farm owners, educated supervisors and some degree of knowledge about antimicrobial resistance and susceptibility testing. Finally, flocks F22 and F23 clustered together in all weeks, and in the MCA corresponded to the quadrant of flocks with full vaccination status, farmers with high education levels, biosecurity measures in place and trained supervisors.

Notably, flocks with high mortality and inconsistent antibiotic usage such as F2, clustered separately in weeks 3 to 5, suggesting ineffective disease control. Interestingly, some high-performing flocks (F7 and F12) with strong high weight gains also clustered apart due to high mortality, indicating that weight alone is not an indicator of flock health.

Flock-level mortality-weight relationship and Feed Conversion Ratio (FCR): A total of 26 broiler flocks comprising 0.618 million birds were evaluated for production performance.

The average flock mortality ranged from 1.09% to 22.4% while FCR values varied between 1.54 to 1.72 among completed cycles (Table 3). Linear regression identified FCR as a significant predictor of mortality percentage ($\beta = 20.5$, $p < 0.0001$). Flocks with higher FCRs consistently showed elevated mortality, highlighting the link between feed efficiency and flock health, suggesting that higher mortality in the flocks can be due to underlying disease pressure, but also due to environmental stress (Figure 4 A & B).

The results on disease occurrence show that particularly bronchitis affected flocks at a higher live weight (Figure 4A) and was present in flocks with high mortality later in the cycle (Figure 4B), which is possibly due to environmental stress. These results highlight the interrelatedness between mortality and underlying management factors which require flock-specific strategies to overcome mortality without affecting growth performance.

Antibiotic usage trends during different stages of production and diseases: Figure 5 shows the different classes of antibiotics used in poultry flocks. The number of antibiotic classes used in flocks ranged from 2-7 with highest observed diversity in flocks F3 and F21, each receiving antibiotics from 7 and 5 different classes, respectively, including polymyxins, sulfonamides, tetracyclines, macrolides and quinolones. Notably, polymyxins (i.e., colistin) were commonly used in several (76.92%) flocks, despite their classification as last resort antibiotics for prophylaxis and treatment of flocks, and their importance for human treatment.

The second highest used antibiotic class was quinolones (65.38%), followed by aminopenicilins (53.84%; amoxicillin). In addition, macrolides (34.61%) and aminoglycosides (30.76%) were also used by farmers. The antibiotic classes with the lowest use were sulfonamides (15.38%), tetracyclines (11.53%) and nitrofuran (3.84%).

Among the 26 flocks, antibiotics were administered 40 times in total without any diagnosed infection or disease, indicating routine prophylactic use (Figure 6A). Week 1 alone accounted for 42.5% (n= 17/40) of that antibiotic usage, indicating a clear bias toward prophylactic usage in early chicks. In contrast, flocks diagnosed with enteritis contributed 16% of total antibiotic use, with peak usage observed in week 4. Other diseases (9%), bronchitis (3%) and Newcastle disease (2%) contributed less to antibiotic usage (Figure 6A). These observations show that the presence of disease only partially explains the observed variation in antibiotic usage, suggesting other influencing factors such as preventive practices, farm management strategies or seasonal trends may also play a role.

Antimicrobial supplements were mostly used to prevent infections in the flocks (Figure 6B), with no clinical disease (n =43). Unlike antibiotics, supplement use in healthy flocks peaked in week 2 (35%) and was more evenly distributed during the later stages of production (week 3-5), implying a more sustained but generalized approach to flock supplementation. Flocks with enteritis received 12.1% of antimicrobial supplements, predominantly during weeks 3 and 4, while flocks with Newcastle disease and flocks with bronchitis received a minimal amount (6% and 4.5% of the total usage, respectively).

Antibiotic usage patterns were monitored across flocks during week 1 to week 5 of the broiler production cycle. The data revealed that antibiotics were most frequently administered during the first and third week of production. As a prophylactic measure, Most farmers used antibiotics during the first week of life (88.46%). The usage of antibiotics then decreased to 42.30% in the second week, 57.69% in the third week, 50% in the 4^{th} week, and 30.43% in 5^{th} week (Figure 7).

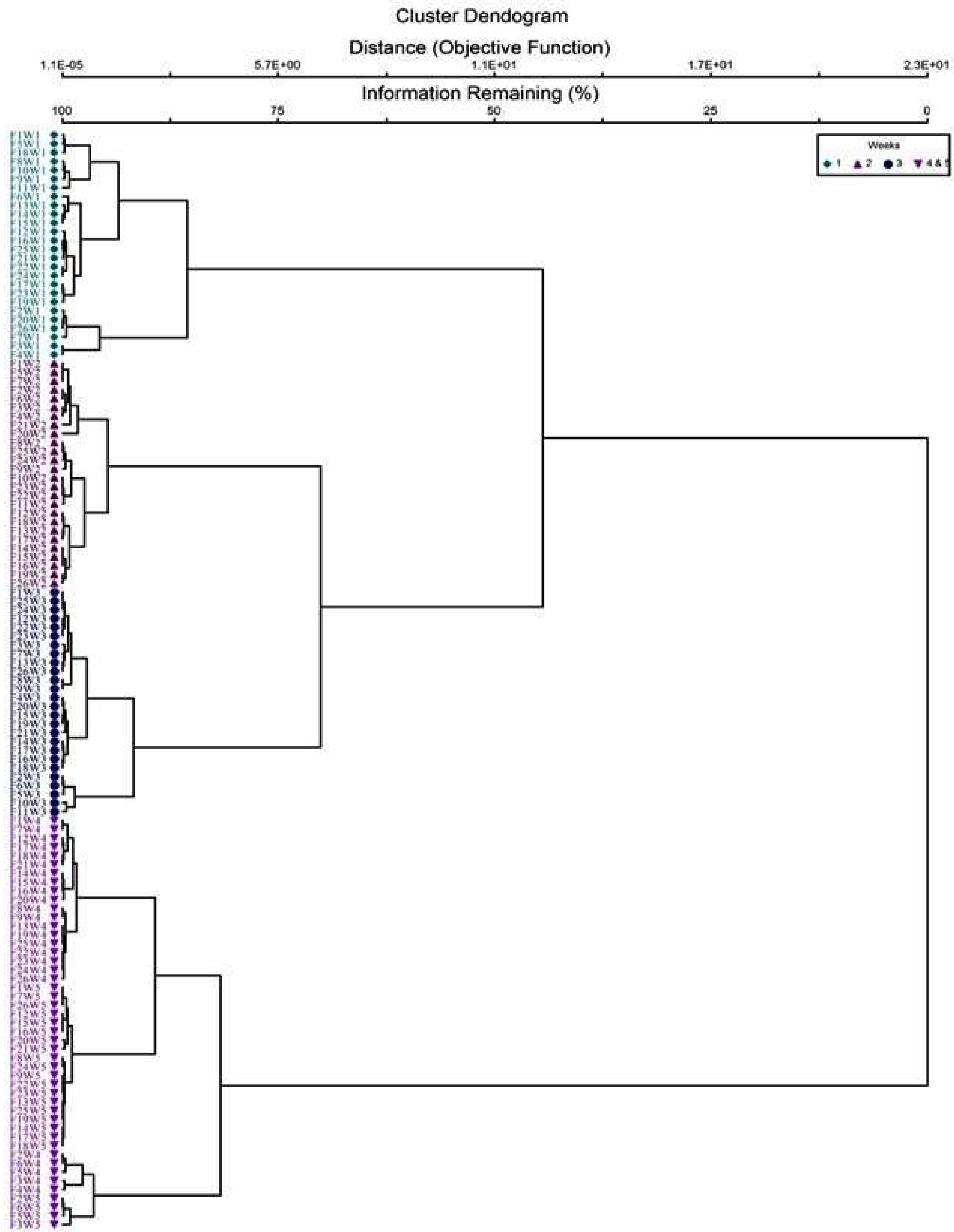

Figure 3. Cluster dendrogram of farms based on weekly production and medication data

Table 3. Summary of flock size, average body weight, mortality percentage and feed conversion ratio (FCR) for 26 commercial broiler flocks.

Flock ID	Flock Size	Average Weight	Mortality %	Feed Conversion Ratio (FCR)
Flock1	22000	2000	2.40	1.63
Flock2	30000	1930	9.5	1.66
Flock3	30000	1825	5.36	1.65
Flock4	25000	1370	5.04	Culled
Flock5	30000	1910	9.05	1.61
Flock6	30000	1865	9.23	1.61
Flock7	30000	1967	2.4	1.58
Flock8	3000	1810	6.6	1.66
Flock9	3000	1860	7.1	1.59
Flock10	3000	700	17.83	Culled
Flock11	3000	685	22.4	Culled
Flock12	30000	1895	1.59	1.54
Flock13	30000	1865	1.56	1.66
Flock14	30000	1887	2.42	1.56
Flock15	30000	1847	2.73	1.64
Flock16	30000	1865	2.37	1.72
Flock17	30000	1901	1.75	1.57
Flock18	30000	1921	1.58	1.62
Flock19	30000	1845	1.85	1.59
Flock20	30000	1978	4	1.6
Flock21	30000	1850	2.88	1.58
Flock22	30000	1900	1.18	1.56
Flock23	30000	1890	1.09	1.55
Flock24	30000	1810	1.38	1.62
Flock25	30000	1865	1.43	1.56
Flock26	30000	1948	2.28	1.59

Culled=There was an outbreak in the flock, so it was removed.

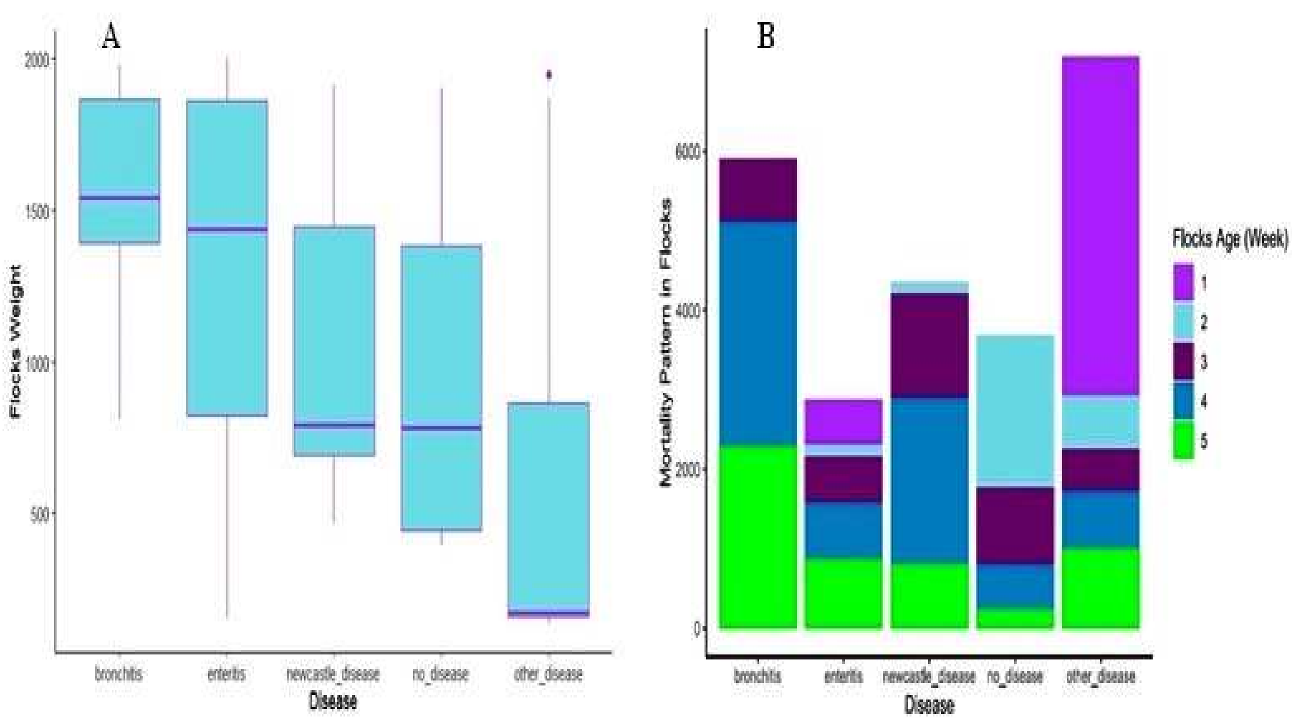

Figure 4. Box plot of flock weights (A) and weekly mortality (B) in flocks with occurrence of various diseases

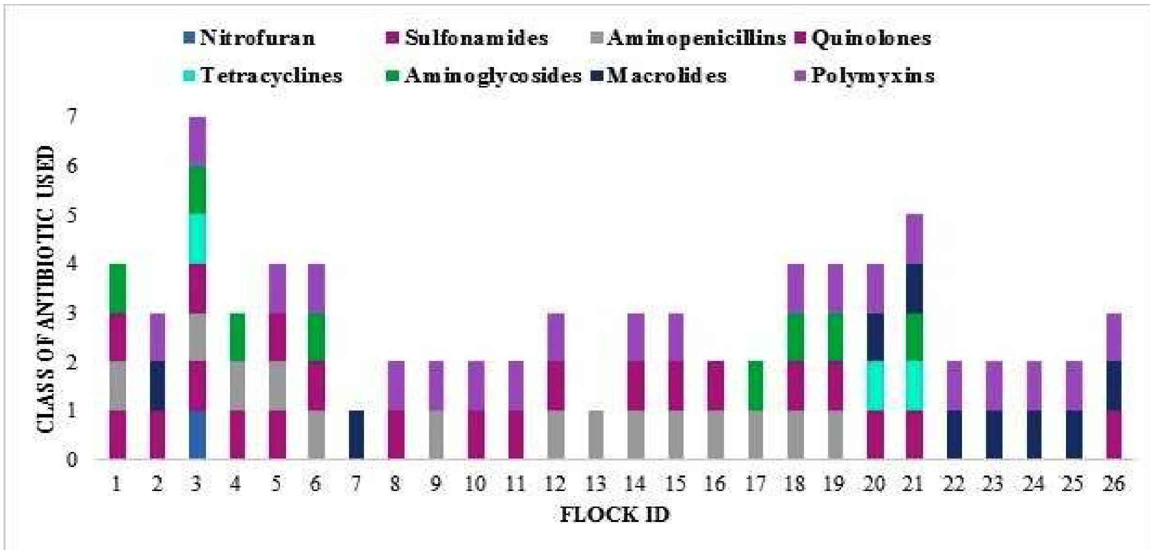

Figure 5. Various antibiotic classes used across different farms.

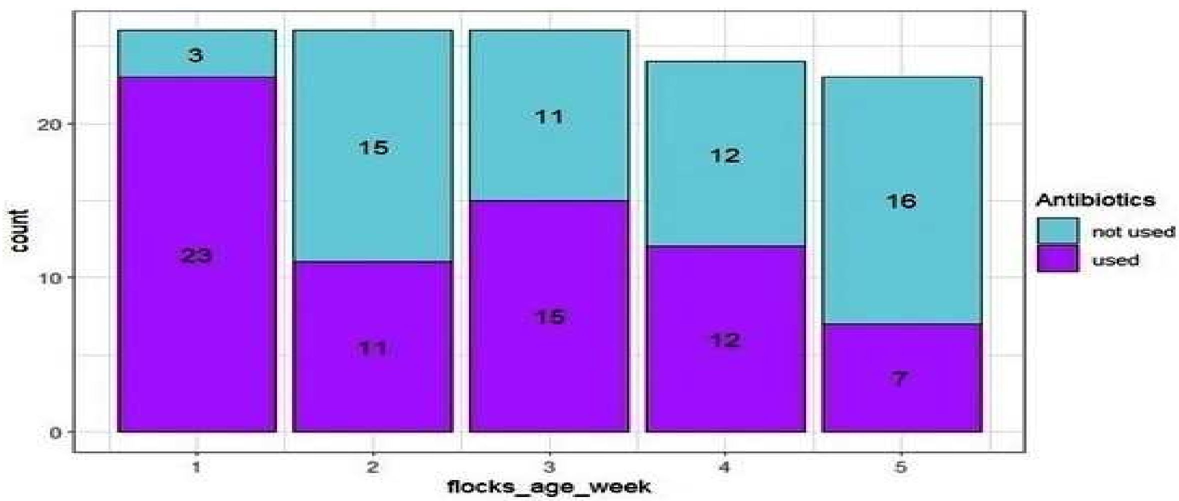

Figure 6. Antibiotics (A) and antimicrobial supplements (B) are used in flocks during different weeks and for different diseases

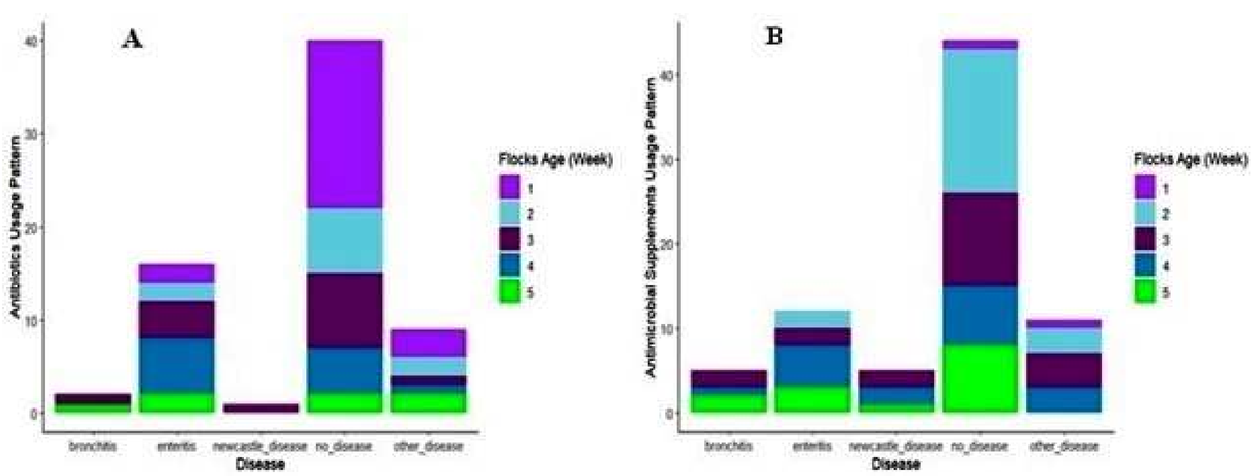

Figure 7. Number of farmers who used antibiotics at different stages of their flocks

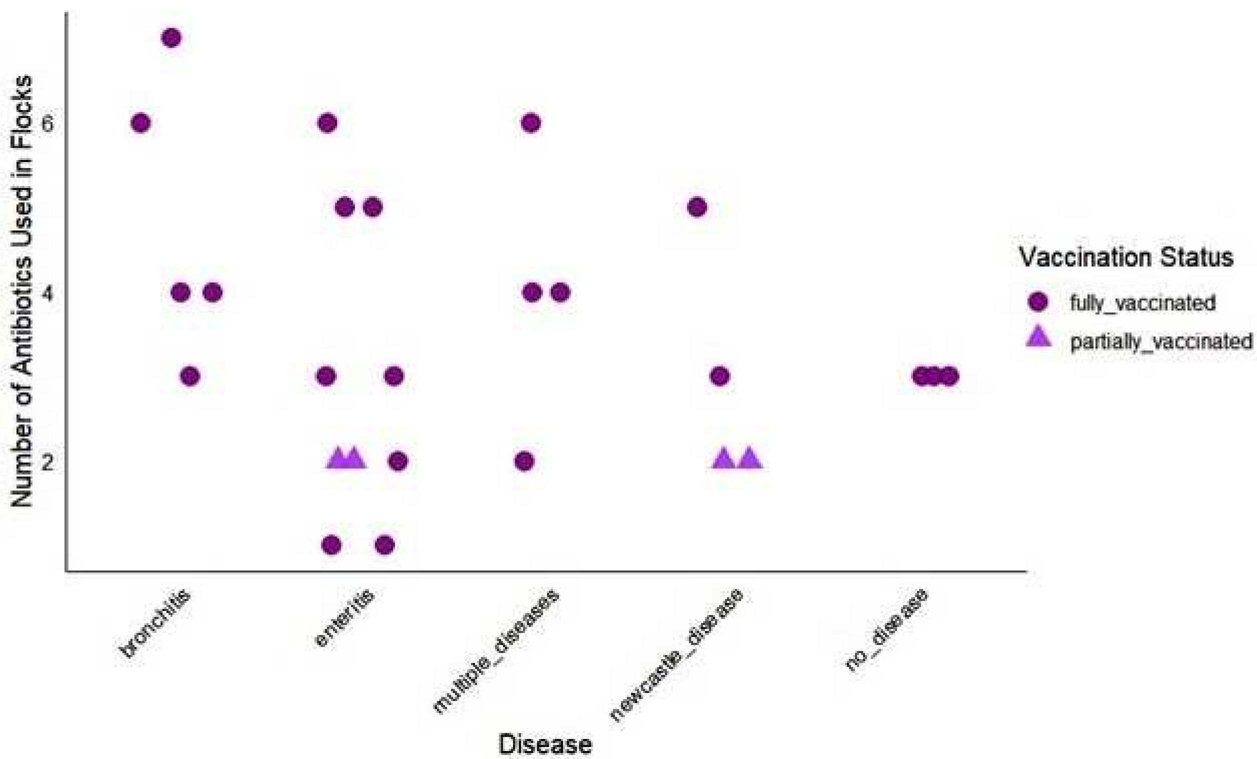

Figure 8. Number of antibiotics used in relation to disease and vaccination status of the flocks

The usage of antibiotics in different flocks in relation to the vaccination status and the occurrence of various diseases was also investigated (Figure 8). During the occurrence of bronchitis and Newcastle disease, in fully-vaccinated flocks, several farmers used more than two antibiotics. Particularly for flocks with enteritis, farmers tended to use multiple antibiotics for treatment. Interestingly, in healthy flocks, farmers used three antibiotics most likely for prophylactic purposes.

DISCUSSION

Our investigations highlight the pattern of antibiotic usage, disease prevalence, weight gain and mortality within the broiler flock production of Rawalpindi and Khyber Pakhtunkhwa regions of Pakistan. This study collected data across all five weeks of the production cycle of 26 flocks, providing a detailed view of the dynamics of broiler production. Notably, three farmers were impelled to cull their infected flocks due to a Newcastle disease infection. We identified a relationship between weight gain and mortality, disentangling the link between collective flock health and productivity. These findings have already been reported in a previous study (Umair *et al.,* 2021). The cluster dendrogram analysis showed four distinct groups based on the weekly measured variables weight gain, mortality, disease occurrence, and antibiotic medication. The clusters mostly showed a separation by production week, however some flocks consistently clustered together throughout the production cycle, showing similarities in production indicators, health status and level of antibiotic treatment. The same flocks have grouped together in the MCA analysis, due to variables related to vaccination and biosecurity status, and the levels of education, training, farming experience and knowledge about antimicrobial resistance of farm owners and supervisors. This highlights the urgent need for farmers' proper education and training of farm supervisors in the topic of antimicrobial resistance, as vital for ensuring sustainable production practices.

Our results also shed light on a severe issue: the prescription and usage of antibiotics in Pakistan are insufficiently regulated, especially in the livestock and poultry sectors, where extensive gaps exist. This lack of regulation and management is an essential factor contributing to the vast extra-label use of antibiotics. Misuse of antibiotics has been coupled with the development of drug-resistant microbial infections in Pakistan in previous studies (Nisar *et al.,* 2017; Rahman and Mohsin, 2019).

Alarmingly, our research work found that antibiotic consumption in broilers is highest during the first week of the rearing cycle, initially for disease prevention, with over 80% of the participating farmers applying antibiotics to their broiler farms. Among the antibiotics, enrofloxacin and colistin were the most frequently used, antibiotics reportedly involved in the emergence of superbugs (Guetiya Wadoum *et al.,* 2016; Wongsuvan *et al.,* 2018). The use of colistin, a last-resort antibiotic, was particularly high (76.92%), similarly to earlier findings of colistin use in Pakistan (Habiba *et al.,* 2023). Colistin use was followed by the use of quinolones (65.38%) and amoxicillin (53.84%), also in accordance to what was observed in previous studies carried out in Pakistan (Umair *et al.,* 2021). This is compared with other countries, where antibiotic usage in flocks was reported as 76% in Europe (Joosten *et al.,* 2019), 61% in Belgium (Persoons *et al.,* 2012), 63% in Thailand (Wongsuvan *et al.,* 2018) and 36% in Vietnam (Cuong *et al.,* 2019). However, it is important to note that the reported figure for Europe largely reflects historical data of therapeutic usage under veterinary supervision. Importantly, however, Regulation (EC) No 1831/2003 of the European Union banned the use of antibiotics as growth promoters in animal feed at the beginning of January 1, 2006. Since then, antibiotic use in poultry has significantly declined in many EU countries, particularly for non-therapeutic purposes (Anonymous 2005). The European Medicines Agency 2023 reported that antibiotic sales for food-producing animals in the EU declined by 53% from 2011 to 2022, with the sharp reduction in Category B (high-risk) antimicrobials, while Category D (first-line treatments) continues be widely used (Anonymous 2023). The overuse of colistin in poultry flocks is notably concerning, giving its crucial importance in controlling and treating gram-negative bacterial diseases in human beings. In our study, colistin was the most commonly used antibiotic in the flocks. Colistin resistance has been reported earlier in *E. coli* from broiler meat Pakistan as 26% (Noreen *et al.,* 2022), is South Asia as 30% (Dawadi *et al.,* 2021) and 41.1% in Egypt (Badr *et al.,* 2022). The overuse of colistin contributed to the emergence of colistin resistance genes (mcr-family) (Liu *et al.,* 2016), which represent an emerging threat that could critically limit treatment choices for superbug infections in humans. Urgent regulatory measures are mandatory to restrain the use of colistin in the poultry industry of Pakistan to safeguard public health.

Our research work also describes that antibiotics are mostly used for prophylactic measures, and during the occurrence of different diseases including enteritis, other diseases (yolk sac infection, mycoplasma, early chick to mortality), Newcastle disease and bronchitis. Although previous studies have reported Newcastle disease and bronchitis as the most prevalent health issues in commercial poultry flocks (Umair *et al.,* 2021). Our findings showed that enteritis was the most frequently observed condition in the sampled broiler farms, followed by Newcastle disease and bronchitis. Shockingly, some farmers rely on their personal experiences or their farm manager or supervisor rather than on a veterinarian's assessment for antibiotic use. Such practices lead to the administration of inadequate doses, incorrect drug combinations and incompatible choice of drugs for the treatment of an infection and improper adherence to the withdrawal period. Such practice combined with direct purchase of antibiotics from different dealers contributes to the overuse and misuse of antibiotics, which inevitably leads to the increase in antibiotic-resistant bacteria. This problem is prevalent in other countries of the world with low and middle income (Braykov *et al.,* 2016; Oberoi *et al.,* 2016; Al-Mustapha *et al.,* 2020). The violation of the withdrawal period is among the most critical practices contributing to the public health risk of antimicrobial resistance due to foodborne transmission, as indicated in previous studies (Guetiya Wadoum *et al.,* 2016; Al-Mustapha *et al.,* 2020). Although most farmers have applied good biosecurity practices on their poultry farms, the waste management was improper and inadequate. Bedding materials contaminated with antibiotics excreted in feces are used as manure, increasing the risk of environmental contamination with antibiotics and multidrug-resistant bacteria (Koutsoumanis *et al.,* 2021). The association between animal feces and the spread of antimicrobial resistance, which was discussed in our study, is well supported in previous literature ((Yeom *et al.,* 2017; Masood *et al.,* 2023).

Our results show that flocks were generally healthy during the first two weeks of their production cycle. A previous study (Mahmood *et al.,* 2024) described that due to prophylactic use of antimicrobial treatments, most of the flocks were healthy during these critical stages of life. Moreover, bronchitis infection, Newcastle disease and enteritis were common after the second week. Enteritis affected the flock all over the production cycle, with high feeding rate and associated metabolic issues likely contributing to this condition. These findings emphasize the importance of proper antibiotic use, improved flock management practices, farmer education and training and implementation of policies through effective legislation, all of which are essential for reducing the risk of illness, improving flock health and productivity and most importantly, combating antibiotic resistance.

Conclusion: This study shows that among intensive broiler farming, an important player in the poultry industry of Pakistan. Husbandry practices differ from farmer to farmer. To maintain infection control, the farmers usually use antibiotic combinations spanning several antibiotic classes. Based on owner-reported data, antibiotic usage in most flocks was not linked to clinical diagnosis, highlighting the need for improved diagnostic support and stewardship in poultry production systems. Our findings describe the strength of educational interventions and farming-oriented training in improving farm production and management strategies for sustainable farming practices and ensuring food safety and security.

Funding: This project did not receive any specific financial assistance to carry out this work.

Conflicts of Interest: The authors declare that no financial or any other conflicts of interest associated with the manuscript exist.

Data availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' Contribution: **HM** Writing – original draft, Formal analysis, Data curation, Conceptualization **ASRD** Data Analysis, Co-supervision, Resources **SHA** Data Collection, **AA**, **MB** Writing – review & editing, Methodology, **MKS,** **AAS**, Validation, Software, Investigation **SK** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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