Veterinary Integrative Sciences 2025; 23(1): e2025026-1-12. DOI: 10.12982/VIS.2025.026



Veterinary Integrative Sciences ISSN; 2629-9968 (online)



Research article

The use of critically important antimicrobials for human use in poultry farming in Pakistan: A cross-sectional study among different types of farmers

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Abstract

WHO's Global Action Plan on Antimicrobial Resistance (AMR) and Pakistan's National Action Plan on AMR, aimed to phase out the Critically Important Antimicrobials (CIAs) in livestock farming. This cross-sectional study aimed to examine antimicrobial use (AMU) patterns among 386 poultry farms and investigate the association between farming types and AMU patterns in Punjab, Sindh, Balochistan, and Khyber Pakhtunkhwa, and Islamabad Capital Territory province in Pakistan. A questionnaire-based survey revealed that all participants were male, the majority belonged to the middle-income group (46.9%) and a significant proportion was university-educated (39.4%). Extensive use of the Highest Priority CIAs, including enrofloxacin (72.3%), colistin sulfate (61.9%), and tilmicosin (36.3%) was observed. High Priority CIAs, including amoxicillin, gentamycin, and neomycin, were used by 48.4%, 28.2%, and 36.3% of farms, respectively. A chi-square test showed a significant association between farming types and AMU patterns (p<0.05), except for aminoglycosides and doxycycline. Breeders showed the highest antimicrobial usage frequency, while backyard farmers tended to use them infrequently. Broiler and layer farmers occupied intermediary positions between these two extremes. Among various farming, broilers exhibited the highest prevalence of preventive AMU (47.8%). Breeders exhibited the lowest frequency of either combination therapy or whole flock treatment when few got sick at 22.2% and 44.4%, respectively. Our findings showed extensive CIA utilization in poultry farming. These findings could guide the phasing out process of HtP-CIAs in poultry, and potentially other food animals in Pakistan and policy making.

Keywords: Antimicrobial resistance, Antimicrobial use, Critically Important Antimicrobial, Poultry farming, Pakistan

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Article history;received manuscript: 28 March 2024,
revised manuscript: 20 April 2024,
accepted manuscript: 27 May 2024,
published online: 10 June 2024,Academic editor;Nguyen Trong Ngu

INTRODUCTION

The widespread antimicrobial use (AMU) in humans and animals leads to antimicrobial resistance (AMR), which has emerged as a significant challenge to public health worldwide (Umair et al., 2022). To combat the growing AMR, the World Health Organization (WHO) does not recommend AMU for healthy animals owing to their misuse and overuse in food production (Lindmeier, 2017). The WHO lists numerous Critically Important Antimicrobials (CIAs) for human health that are widely used in veterinary care and are essential for treating severe bacterial infections in humans, for which currently few or no alternatives exist (World Health Organization, 2019). Antibiotic growth promoters (AGPs) were first introduced in Europe, but this misconduct was rectified in 2006 by banning it. However, the state of affairs regarding AGPs in Asia, Africa, and Latin America is more concerning with many countries in these regions still permitting their use (European Commission, 2005).

With approximately 33,331 poultry farms dispersed across Pakistan, the country has emerged as the 11th largest poultry producer globally, accounting for 40–45% of total meat consumption. According to Pakistan's last livestock census, there were 110 breeders, 29,889 broilers, and 3,332 layer farms recorded (Pakistan Bureau of Statistics, 2006; Kamal, 2023). By 2023, it was estimated that the backyard poultry population reached around 94 million, while commercial poultry numbered approximately 1,792 million birds. The commercial poultry sector included layers, broilers, and breeder stock, with an estimated population of 73 million, 1,703 million, and 16 million birds, respectively (Ministry of Finance, 2023). Over the past decade, the industry has shown remarkable growth, expanding at an approximate rate of 7.3% annually (Ministry of Finance, 2022).

As the poultry sector grows, AMU intensifies to substitute for proper hygiene and augment production to meet growing demand (Umair et al., 2022). Pakistan has a high level of AMU in poultry, with a rate of 35.4 mg/population correction unit (PCU) at the end of last decade, which is expected to increase by 44% by the end of the current decade, thus turning Pakistan into global AMU hotspot (Mulchandani et al., 2023). In Pakistan, about half of AMU in food animals comes from the Highest Priority Critically Important Antimicrobials (HtP-CIA), and commercial farms are consuming a large chunk of them (Mohsin and Umair, 2020; Umair et al., 2021). Further, about eight hundred tons of the seven HtP-CIA, with a corresponding consumption of 10.05 mg per unit of animal biomass, was observed (Umair et al., 2022). It has also been reported that CIAs were regularly prescribed by veterinarians in Pakistan (Saman et al., 2023).

In low-middle-income countries (LMICs), the incorrect and irresponsible AMU in poultry farming has become a widespread problem (Islam et al., 2022). Regarding high antimicrobial consumption, Pakistan is the third among LMICs (Atif et al., 2019). The annual consumption of antibiotics in broiler farming in Pakistan might be about 568 tons (Mohsin et al., 2019). A recent study reported that about 73% of chicken meat samples have some antimicrobial activity, indicating a high prevalence of antimicrobial residues (Ali and Saleem, 2020). Multiple studies have provided evidence of the circulation of antimicrobial-resistant pathogens in poultry in Pakistan, carrying antimicrobial resistance genes (ARGs) that are of zoonotic significance. Previous published works have confirmed the resistance of these strains to various antimicrobials (Azam et al., 2017; Nisar et al., 2017; Kamboh et al., 2018; Waseem et al., 2019; Dawadi et al., 2021). Yearly, on-farm use of antibiotics was about 251 mg/kg of the final flock weight, which was second only to China (Mohsin et al., 2019). AMR's public health impacts were grimmer than those indicated in numerous published works in recent decades (Ministry of National Health Services, 2017). The problem is further compounded by a lack of proper regulation and oversight concerning the AMU in poultry farming, especially in rural areas. The unregulated and nonprescription AMU may aid in the rise of



drug-resistant pathogens, thus increasing the incidence of AMR (Habiba et al., 2023).

Farm biosecurity and AMR awareness are critical determinants of the rational administration of antimicrobials (Habiba et al., 2023). Monitoring AMU is essential for mitigating the dissemination of AMR (Umair et al., 2022). Researching existing practices and usage of antimicrobials is part of the priority areas of Pakistan's National Action Plan on AMR (Ministry of National Health Services, 2017). It is critical to phase out the use of CIAs in the poultry sector to contain the AMR threat (Habiba et al., 2023). There is a lack of studies on the use of CIAs in Pakistan's poultry sector; hence, this study was conducted to assess the pattern of AMU in Pakistan's poultry sector and to identify the HtP-CIAs used in poultry farming. Additionally, the association between the farming types and AMU patterns, multiple AMUs, and critical practices regarding AMU were determined. The findings of this study could be used in the phasing out process of WHO's HtP-CIA use in food animals, especially in poultry in Pakistan. These findings serve as a foundational framework for future inquiries in this domain.

MATERIALS AND METHODS

Study design

A cross-sectional study was formulated to evaluate the general information and the antibiotic usage patterns among poultry farmers across all four provinces of Pakistan namely Punjab, Sindh, Khyber Pakhtunkhwa and Balochistan, encompassing the Islamabad Capital Territory (ICT) region. The study was conducted from August to October 2023.

Study population and sample

The study encompassed poultry farmers engaged in diverse farming practices, including broiler, layer, breeder, and backyard poultry, across multiple provinces in Pakistan, including Punjab, Sindh, Khyber Pakhtunkhwa (KPK), Baluchistan, and the ICT. The sample size was calculated based on single proportion estimation with a 95% confidence interval and 5% margin of error. Based on the assumption of maximum variability, antimicrobial usage on flocks in poultry farmers was set at 50% (p = 0.5). The estimated sample size was at least 385; however, the study ultimately included 386 poultry farmers practicing various farming methods across Pakistan. Inclusion criteria were poultry farmers aged 18 and above who are managers or owners of their farms and have at least one year of experience. Farmers who raised poultry for show or game purposes, as well as pet birds, were excluded. A convenient sampling technique was employed, and farmers were selected from different districts with the assistance of field veterinarians, poultry veterinarians, personal contacts, and through a door-to-door survey.

Questionnaire development, validation, and data collection

The questionnaire items were developed after taking insight from previous studies (Nuangmek et al., 2018; Hassan et al., 2021; Nohrborg et al., 2022; Habiba et al., 2023). Three experts validated the questionnaire. This validation ensured questionnaire consistency and relevance for the study's intended goals. The questionnaire was translated into Urdu, and then back to English. It was tried out on 20 participants and modified based on the results of the pretesting (Tsang et al., 2017).

A well-structured questionnaire was used to collect demographic data on farmers, farm characteristics, biosecurity measures, training engagement, access to veterinary care, AMR information, antimicrobial usage patterns, and critical



practices regarding AMU. AMU patterns were determined using Yes/No questions about specific antimicrobials, with frequency of use measured on a four-point Likert scale (rarely, sometimes, often, and always). Critical practices were questioned regarding normal actions including preventive use of antimicrobials, combination therapy with multiple antimicrobials, and whole flock treatment when few get sick.

The samples include any person rearing poultry in Pakistan based on a convenience sampling technique. We visited the farmers in person to collect the questionnaire-based data. If someone was not approachable in person for any reason, such as biosecurity, we opted to do the questionnaire online.

Data analysis

Data were analyzed using the IBM Statistical Package for Social Sciences (SPSS) Version 29 (IBM Corporation, Armonk, New York, USA). Descriptive statistics were used to calculate the frequencies and percentages of different variables. The chi-square test was used to investigate the association between farming types and AMU patterns, multiple antimicrobial usage, and critical practices regarding AMU.

Ethical consideration

Ethical clearance was obtained from the Faculty of Public Health, Chiang Mai University human research ethics committee, via document No ET031/2023. Before data collection, the aims and objectives of our study were explained to our participants, and informed consent was sought.

RESULTS

Farming characteristics and sociodemographic composition of the farmers

Three hundred eighty-six poultry farmers were enrolled in this study, including 226, 109, 27, and 24 farmers from broiler, layer, breeder, and backyard farming, respectively. Almost all participants were male (99%). Of the participants, 11.4% completed primary education, 31.1% completed secondary education, 12.0% completed intermediate education, and 39.4% completed graduation or above. On the other hand, 3.1% of the farmers were illiterate. As far as the province-wise recruitment of participants is concerned, Punjab, Islamabad, Sindh, Khyber Pakhtunkhwa, and Baluchistan made up 62.0%, 13.5%, 11.0%, 8.6%, and 4.9% of participants, respectively. About half of the farmers were middle-income, while the high-income group comprised about a guarter. The mean of experience in poultry farming was 11.16±8.05 years. Half of the participants were owners of poultry farms, about a quarter were managers or supervisors, and the rest had a dual role. Most farmers belong to non-corporate sectors, while around 16.0% (n=61) belonged to the corporate sector. Small, medium, and large-scale farmers comprised about 14%, 41%, and 45%, respectively. Most farmers (68%, 262 out of 386) had an all-in, all-out production system, and about a quarter had no farm biosecurity measures. Many farmers had no training concerning AMU use in poultry farming, as only 31% (n=120) reported having some AMU training in poultry and poultry production. Most farmers reported that they could access veterinary services easily, and around 44% had no information related to AMR, as shown in Table 1.



Table 1 Characteristics of poultry farmers in Pakistan (n=386)

Variable	Broiler	Layer	n (%) Breeder	Backyard	Overall			
	(n=226)	(n=109)	(n=27)	(n=24)	(n=386)			
Age (Mean±S.D.)	38.91±9.30	38.40±10.23	35.70±8.70	41.46±16.42	38.70±10.13			
Educational Level								
Illiterate	7 (3.1)	3 (2.8)	0 (0.0)	2 (8.3)	12 (3.1)			
Primary	33 (14.6)	8 (7.3)	0 (0.0)	3 (12.5)	44 (11.4)			
Secondary	76 (33.6)	36 (33.0)	3 (11.1)	5 (20.8)	120 (31.1)			
Intermediate	39 (17.3)	14 (12.8)	1 (3.7)	4 (16.7)	58 (15.0)			
Graduation	71 (31.4)	48 (44.0)	23 (85.2)	10 (41.7)	152 (39.4)			
Province (n=384)								
Islamabad	14 (6.3)	26 (23.9)	4 (14.8)	8 (33.3)	52 (13.5)			
Punjab	142 (63.4)	64 (58.7)	21 (77.8)	11 (45.8)	238 (62.0)			
KPK	29 (12.9)	1 (0.9)	2 (7.4)	1 (4.2)	33 (8.6)			
Sindh	31 (13.8)	10 (9.2)	0 (0)	1 (4.2)	42 (10.9)			
Balochistan	8 (3.6)	8 (7.3)	0 (0)	3 (12.5)	19 (4.9)			
Family income per month (Ru	pees)							
<40,000	67 (29.6)	25 (22.9)	1 (3.7)	12 (50.0)	105 (27.2)			
40,000-100,000	111 (49.1)	52 (47.7)	10 (37.0)	8 (33.3)	181 (46.9)			
>100,000	48 (21.2)	32 (29.4)	16 (59.3)	4 (16.7)	100 (25.9)			
Experience (Mean±S.D.)	11.84±7.56	11.15±8.75	10.52±9.18	5.39 ± 5.50	11.16±8.05			
(n=378)								
Position (n=385)								
Owner	117 (52.0)	65 (59.6)	4 (14.8)	17 (70.8)	203 (52.7)			
Manager	49 (21.8)	33 (30.3)	22 (81.5)	3 (12.5)	107 (27.8)			
Both	59 (26.2)	11 (10.1)	1 (3.7)	4 (16.7)	75 (19.5)			
Farm ownership (n=384)								
Non-corporate	205 (91.1)	89 (81.7)	6 (22.2)	23 (100)	323 (84.1)			
Corporate	20 (8.9)	20 (18.3)	21 (77.8)	0 (0)	61 (15.9)			
Flock size (n=385)								
≤1000	18 (8.0)	12 (11.1)	1 (3.7)	22 (91.7)	53 (13.8)			
1001-5000	114 (50.4)	41 (38.0)	2 (7.4)	2 (8.3)	159 (41.3)			
>5000	94 (41.6)	55 (50.9)	24 (88.9)	0 (0.0)	173 (44.9)			
Production system								
All in out	171 (75.7)	66 (60.6)	21 (77.8)	4 (16.7)	262 (67.9)			
Continuous	40 (17.7)	35 (32.1)	5 (18.5)	19 (79.2)	99 (25.6)			
Both	15 (6.6)	8 (7.3)	1 (3.7)	1 (4.2)	25 (6.5)			
Biosecurity measures	146 (78.5)	55 (59.1)	25 (96.2)	11 (47.8)	237 (72.3)			
(n=328)								
Training	60 (26.5)	34 (31.2)	23 (85.2)	3 (12.5)	120 (31.1)			
Ease of access to	193 (85.4)	97 (89.0)	23 (85.2)	16 (66.7)	329 (85.2)			
veterinary service		/						
AMR information	119 (52.7)	62 (56.9)	26 (96.3)	8 (33.3)	215 (55.7)			

Source of information of poultry farmers on AMR

The majority of AMR information is mostly private veterinarians (56.3%), followed by education and training (34.9%), government veterinarians (30.7%), social media (18.6%), electronic media (18.1%), and print media (14.0%), as shown in Table 2.

Table 2 Source of information on AMR (n=215)

Source of information	Frequency (n)	Percent
Print media	30	14.0
Electronic media	39	18.1
Private veterinarians	121	56.3
Government veterinarians	66	30.7
Social media	40	18.6
Education and training	75	34.9

AMU patterns

In investigating antimicrobial usage in poultry farming, varying patterns across ten selected antimicrobials were revealed for this study. The most



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commonly utilized antibiotic was enrofloxacin (72.3%), which was sometimes used in 53% of cases, often used in 21.3%, and rarely used in 20.8% of cases. Colistin sulfate was being used by 62% of farmers, with 46.4% using it sometimes. Amoxicillin, doxycycline, tilmicosin, and neomycin were reported to be used in 48.4%, 47.2%, 36.3%, and 36.3%, respectively. Furaltadone had the lowest use (18.0%), as shown in Table 3.

 Table 3 Frequency of antibiotics used among poultry farmers in Pakistan (n=386)

Class	Class Antibiotic No. of use, n Frequency of use, n				y of use, n (%)	
		(%)	Always	Often	Sometimes	Rarely
Quinolones	Enrofloxacin 1	279 (72.3)	14 (5.0)	59 (21.1)	148 (53.0)	58 (20.8)
Polymyxin	Colistin ¹	239 (61.9)	17 (7.1)	67 (28.0)	111 (46.4)	44 (18.4)
Macrolides	Tilmicosin 1	140 (36.3)	5 (3.6)	29 (20.7)	60 (42.9)	46 (32.9)
Penicillin	Amoxicillin ²	187 (48.4)	14 (7.5)	44 (23.5)	78 (41.7)	51 (27.3)
Aminoglycosides	Gentamycin ²	110 (28.2)	3 (2.7)	15 (13.6)	43 (39.1)	49 (44.5)
	Neomycin ²	140 (36.3)	10 (7.1)	29 (20.7)	57 (40.7)	44 (31.4)
Tetracycline	Doxycycline ³	182 (47.2)	11 (6.0)	32 (17.6)	77 (42.3)	62 (34.1)
	Oxytetracycline ³	128 (33.2)	5 (3.9)	15 (11.7)	54 (42.2)	54 (42.2)
	Chlortetracycline ³	87 (22.5)	4 (4.6)	14 (16.1)	40 (46.0)	29 (33.3)
Nitrofurans Derivatives	Furaltadone ⁴	70 (18.1)	4 (5.7)	9 (12.9)	24 (34.3)	33 (47.1)

¹ Highest Priority Critically Important Antimicrobial (HtP-CIA)

² High Priority Critically Important Antimicrobial (HP-CIA)

³ Highly Important Antimicrobial (HIA)

⁴ Important Antimicrobial (IA)

Association between type of farming and AMU patterns

The use of antimicrobials including enrofloxacin, colistin, tilmicosin, amoxicillin, furaltadone, oxytetracycline, and chlortetracycline was significantly associated with the type of aminoglycosides class and doxycycline of tetracycline class exhibited no significant association with the type of farming. Enrofloxacin, colistin, and tilmicosin were found to be particularly common in breeder farming, with corresponding frequencies of 81.5%, 85.2%, and 63.0%. Conversely, these HtP-CIAs were least utilized among backyard farmers except for enrofloxacin. The highest use in breeder farming was also observed for amoxicillin, furaltadone, oxytetracycline, and chlortetracycline. The data were shown in Table 4.

Table 4 Association between types of farming and AMU patterns

Class	Antimicrobials	Frequency of use, n (%)				p-value*
		Broiler	Layer	Breeder	Backyard	
Quinolones	Enrofloxacin	175 (77.4)	67 (61.5)	22 (81.5)	15 (62.5)	0.009
Polymixin	Colistin	142 (62.8)	67 (61.5)	23 (85.2)	7 (29.2)	0.001
Mecrolides	Tilmicosin	89 (39.4)	29 (26.6)	17 (63.0)	5 (20.8)	0.001
Penicillin	Amoxicillin	107 (47.3)	51 (46.8)	20 (74.1)	9 (37.5)	0.037
Aminoglycosides	Gentamycin	59 (26.1)	31 (28.4)	13 (48.1)	7 (29.2)	0.124
Aminoglycosides	Neomycin	86 (38.1)	34 (31.2)	14 (51.9)	6 (25.0)	0.128
Nitrofurans	Furaltadone	36 (15.9)	17 (15.6)	11 (40.7)	6 (25.0)	0.010
Tetracycline	Doxycycline	113 (50.7)	48 (44.4)	15 (55.6)	6 (26.1)	0.101
Tetracycline	Oxytetracycline	66 (29.2)	40 (36.7)	16 (59.3)	6 (25.0)	0.011
Tetracycline	Chlortetracycline	51 (22.6)	18 (16.5)	12 (44.4)	6 (25.0)	0.021

*The significance level is 5%, and the p-value is from the Chi-square test

Number of AMU among different types of farming

The results showed a significant association between the type of farming and the number of antimicrobials used in combination or separately at different times (p=0.005). Approximately 60% of backyard farmers were found to utilize fewer than two antimicrobials. Conversely, a similar proportion of breeder farmers reported the usage of five or more antimicrobials. Interestingly more layer farmers (40%) used



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fewer (\leq 2) antimicrobials compared to their counterparts involved in broiler farming (31.4%) and breeder farming (18.5%). Contrarily percentage of broiler farmers using 3-4 antimicrobials was the highest (35.9%) compared to layer (31.5%), breeder (22.2%), and backyard (17.4%) farmers, as shown in Table 5.

Table 5 Association between multiple AMU and types of poultry farmers

Number of antimicrobials	Number of	Type of farming, n (%)				p-value*
	farms, n (%)	Broiler	Layer	Breeder	Backyard	
≤2 antimicrobials	132 (34.6)	70 (31.4)	43 (39.8)	5 (18.5)	14 (60.9)	0.005
3-4 antimicrobials	124 (32.5)	80 (35.9)	34 (31.5)	6 (22.2)	4 (17.4)	
≥5 antimicrobials	125 (32.8)	73 (32.7)	31 (28.7)	16 (59.3)	5 (21.7)	

*The significance level is 5%, and the p-value is from the Chi-square test

Critical practice regarding AMU and its association with the type of farming

A statistically significant association was observed between the critical practice regarding AMU and farming type (p<0.05). The highest prevalence of preventive antimicrobial use was identified among broiler farmers (47.8%), followed by layer, breeder, and backyard farmers, with reported rates of 35.8%, 22.2%, and 16.7%, respectively. Broiler farmers (45.6%) had the highest percentage of combination therapy with multiple antimicrobials to enhance drug efficacy than layer (31.2%), backyard (25.0%) and breeder farmers (22.2%). Additionally, a considerable majority of layer farmers (73.4%) administered treatment to the entire flock when a few get sick, contrasting with a notably lower percentage (44.4%) among breeder farmers, as shown in Table 6.

Table 6 Association between types of farming and clinical practices regarding AMU

Clinical practice regarding	Number of	Number of Type of farming, n (%)				
AMU	farms, n (%)	Broiler	Layer	Breeder	Backyard	
Preventive use of antimicrobials	157 (40.7)	108 (47.8)	39 (35.8)	6 (22.2)	4 (16.7)	0.002
Combination therapy with multiple antimicrobials	149 (38.6)	103 (45.6)	34 (31.2)	6 (22.2)	6 (25.0)	0.007
Whole flock treatment when a few get sick	272 (70.5)	163 (72.1)	80 (73.4)	12 (44.4)	17 (70.8)	0.023

*The significance level is 5%, and the p-value is from the Chi-square test

DISCUSSION

Our investigation revealed that 72.3% of poultry farmers implemented some level of biosecurity measures including controlling the free access to visitors, vehicles, and animals. Similarly, in Nepal, a comparable trend was observed, with 77.5% of farmers reporting the presence of fencing around their farms. However, it was noted that 44% of farmers lacked information regarding AMR, echoing findings from a study conducted in a similar context in Nepal (Subedi et al., 2023). Consistently, studies conducted in Bangladesh also reported similar proportions, as they showed that 40-43% did not know about AMR (Hassan et al., 2021; Islam et al., 2022). However, this contradicts a recent study in Pakistan involving broiler farmers, which reported that around 90% of farmers do not know about AMR (Habiba et al., 2023). Further, the study in Nepal found that 47.62%, 41.04%, and 63.46% of broiler, layer, and backyard farmers, respectively, had no information regarding AMR (Subedi et al., 2023), which is aligned with our results as our results also showed that 47.3%, 43.1% and 66.7% of broiler, layer and backyard farmers respectively lack information on AMR.



The majority of the participants reported that the source of AMR information is primarily private veterinarians (56.3%) and government veterinarians (30.7%), which is evident from other studies as reported by Habiba et al. (2023) about the source of veterinary services in a similar fashion. Moreover, 18.6% of participants claimed that their source of information is social media, an opportunity to help spread AMR information and sensitize poultry farmers regarding the responsible use of antimicrobials in poultry to check further spread of AMR issues. Social media is viral among the youth, which obviously will be the future industry leader in the poultry sector, so social media campaigns may help in future interventions to curtail AMR's growing threat.

The findings which are in line with previous research, support the widespread use of CIAs for human consumption in Pakistan's poultry sector (Mohsin and Umair, 2020; Islam et al., 2022). This is also evident from the fact that CIAs were regularly used by veterinarians, as reported in a recent study about veterinarian's knowledge, attitude, and practice on AMU and AMR in Pakistan (Saman et al., 2023). Our study showed Enrofloxacin and Colistin were the most frequently used HtP CIA antimicrobials. Tilmicosin was an antibiotic that was used less frequently, and our study's observations coincided with their import volume (Umair et al., 2022). However, a different study in Pakistan found that 35.0% of participants used enrofloxacin, which is less than half of the prevalence observed in our study (Habiba et al., 2023). Contrary to their findings, a higher percentage of farmers were observed using chlortetracycline, doxycycline, oxytetracycline, neomycin, gentamycin, and furaltadone. Colistin has been reported to be the most widely used and third most widely used antibiotics in Bangladesh and Nepal, respectively (Islam et al., 2022; Subedi et al., 2023). Almost 48% and 62% of poultry farms in this study were using both amoxicillin and colistin, which aligns with the study which found that 60% of poultry farmers in Pakistan use both of them synergistically (Habiba et al., 2023). Furthermore, a recent study reported that in South Asian developing countries the resistance of E. coli to colistin has reached 30% (Dawadi et al., 2021). Another study has confirmed the presence of colistinresistant mcr-1 gene containing E. coli isolates among poultry in Pakistan (Azam et al., 2017). In addition, Nasar et al. (2017) reported that 84%, and 69%, of Campylobacter spp. isolates (n=58) from poultry meat in Pakistan exhibited high resistance to enrofloxacin, and colistin respectively. Similarly, 82%, 40%, and 8.6% of Campylobacter spp. Isolates were resistant to amoxicillin, neomycin, and doxycycline respectively. These studies validated our study's finding as they identified a comparable resistance pattern to the AMU pattern observed in our study for the same antimicrobials.

In this study, the utilization of all the antimicrobials, except the aminoglycoside class and doxycycline from the tetracycline group, exhibited a significant association with the type of farming (p<0.05). This could be attributed to the affordability of aminoglycosides and doxycycline, potentially resulting in consistent usage across various farming types. Additionally, recall biases among farmers might have limited the scope of our study factor. CIAs such as enrofloxacin, colistin, and tilmicosin were found to be significantly overused in breeder farms, with frequencies of 81.5%, 85.2%, and 63.0%, respectively. The breeder farmers have to maintain not only the health of the breeder flock but also to ensure that the offspring are also healthy and free from vertically transmitted bacterial diseases, and there is also a high prevalence of mycoplasmas and Salmonellosis among breeder (Shoaib, 2020). The breeder farming follows a pyramidal structure in which the grandparent stock (GPS) is at the pyramid's apex, parent stock (PS) lies at the middle, and commercial chickens, which produce eggs or meat for table purposes, lie at the bottom. This hierarchical structure could lead to the fixation of AMR genes and subsequent transfer to the next generation vertically as well as to the human population horizontally through poultry products as well as environmental dissemination, leading to a public health problem (Dierikx et al., 2013; Seo et al.,



2019; Shang et al., 2021). So, this high use among breeder farmers could contribute to the development of AMR across all types of farming (Coupled with less regulation).

Backyard farmers showed the lowest usage of these antimicrobials. This may be because backyard chickens are highly disease-resistant and can perform under harsh environmental and nutritional conditions than broilers and other commercial breeds (Kaleri et al., 2023). Also, we found that the frequency of preventive use was lowest among backyard farmers, which may reflect this phenomenon among backyard farmers. There was a frequent usage of antibiotics among broiler farmers. This might be as a result of our observation that, compared to backyards, they apply antimicrobials for disease prevention at the highest frequency. The higher use among broilers compared to backyard may be because broiler strains are less disease-resistant and more prone to catching infections (Bhuiyan et al., 2019; Korver, 2023). In addition, broilers may need more antibiotics than backyard farmers because they are sold at young ages and are more likely to become sick than mature chicken birds (Gray et al., 2021; Connolly et al., 2022; Korver, 2023).

Our findings suggest that many farmers use antimicrobials for disease prevention on the farms. This misuse might be because there is a lot of disease pressure, and disease plays havoc with farmers' economies in the form of mortality and decreased production. So, farmers resort to antibiotics for preventive purposes to avoid mortality and morbidity, thus maximizing profits. Other studies have reported that fewer farmers use antimicrobials for preventive purposes (Nuangmek et al., 2018; Sattar, 2023). Our finding about antimicrobial combination therapy frequently, which is not considered a good practice except in certain circumstances aligns with the study in Thailand (Nuangmek et al., 2018). On the contrary, a study in Bangladesh reported a much lower proportion of farmers using multiple antimicrobials simultaneously (Islam et al., 2022). Only a fraction of farmers adhered to the excellent practice of never treating the whole flock when a few got sick. Similar results were shown in Bangladesh as they reported that most farmers treat the whole flock when a few get sick (Islam et al., 2022).

The limitations of this study encompass the potential of recruiting individuals non-proportionally across different farming types due to national circumstances, potentially impacting our findings. The convenience sampling techniques used in this study probably resulted in some possible bias due to non-probability, reducing the generalizability of our findings. Additionally, the data collection process involved conducting questionnaire-based interviews for onsite data collection, while online data was gathered through self-reported questionnaires, potentially influencing our findings. Moreover, the cross-sectional study design lacked the capacity to establish a causal relationship between the variables under investigation.

CONCLUSIONS

The trends highlighted by our survey results showed that antimicrobials are being extensively used in poultry production in Pakistan, which needs the immediate attention of policymakers. CIAs of the Highest Priority (enrofloxacin, colistin and tilmicosin) and High Priority (amoxicillin, gentamycin, and neomycin) for treating infections in humans have a relatively high prevalence of use in poultry farming, which needs to be curbed to avoid a public health crisis as it might result in AMR problems. The findings of this study could be used in the phasing out process of WHO's CIAs use in food animals, especially poultry in Pakistan.

ACKNOWLEDGEMENTS

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The authors would like to acknowledge National Reference Lab for Poultry Disease (NRLPD) National Agricultural Research Council Islamabad, Poultry Research Institute (PRI) Rawalpindi, and Poultry Research Institute Mansehra, Pakistan who identified participants and facilitated this survey. We are also grateful to Thailand International Cooperation Agency (TICA) for their support to carry out this study through their Thailand International Postgraduate Program (TIPP) fellowship.

AUTHOR CONTRIBUTIONS

Muhammad Rasheed: Methodology, Investigation, Data analyzing, Writing-original draft, Writing-review & editing.

Warangkhana Chaisowwong: Conceptualization, Methodology, Writing-review & editing.

Parichat Ong-artborirak: Conceptualization, Methodology, Data analyzing, Writing-review & editing.

Aksara Thongprachum: Conceptualization, Methodology, Writing-review & editing

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How to cite this article;

Muhammad Rasheed, Warangkhana Chaisowwong, Parichat Ong-artborirak and Aksara Thongprachum. The use of critically important antimicrobials for human use in poultry farming in Pakistan: A cross-sectional study among different types of farmers. Veterinary Integrative Sciences. 2025; 23(1): e2025026-1-12.

