 doi:10.20944/preprints202207.0402.v1

Article

Patterns and Risk Factors of Antibiotic Use in Poultry Farming and the Farmers: A Cross Sectional One-health Study in Pakistan

Um e Habiba[1*], Amjad Khan[2], Elia John Mmbaga[1] and Muhammad Asadzaman[1*]

[1] Department of Community Medicine and Global Health, Institute of Health and Society, Faculty of Medicine, University of Oslo, Oslo, Norway
[2] Department of Pharmacy, Quaid-i-Azam University, Islamabad, Pakistan
*Corresponding author: u.e.habiba@studmed.uio.no (U.E.H.); muhammad.asaduzzaman@medisin.uio.no ()

Abstract: Antimicrobial resistance (AMR) due to community carriage of antibiotic-resistant bacteria is highly prevalent in the WHO South-East Asia region. One of the major reasons is the misuse of antibiotics in animal farming practices and at community level, which threatens both human and animal health. However, this multifaceted One Health (OH) problem of antibiotic use (ABU) in poultry farms and respective farmers is not well studied in countries like Pakistan. Therefore, we conducted n OH cross-sectional study in rural Punjab to explore the current practices of ABU in poultry and poultry farmers, associated factors, their healthcare-seeking behaviour and biosecurity practices. We found all the participating farmers using antibiotics for poultry, 60% of which were Colistin sulphate and Amoxicillin trihydrate. The significant consumption of antibiotics in poultry farms (60%) and poultry farmers (50%) was without prescription. Most of the farms (85%) had no wastewater drainage system, causing direct shedding of poultry waste and antibiotic residue in the surrounding environment. Lack of farmers' education, professional farm training and duration of farming experience were the significantly associated factors with ABU and knowledge of AMR. Our study implies the necessity of an integrated OH-AMR policy with the inclusion of farmers' education, mass awareness, and strict antibiotic usage guidelines.

Keywords: antimicrobial resistance; One Health; poultry; poultry farmers; antibiotic use; Pakistan

1. Introduction

Misuse of antibiotics in food-producing animal farming practices has become an inevitable challenge to the containment of global antimicrobial resistance (AMR) both in humans and in animals, particularly in the low and middle-income countries (LMICs) [1]. AMR has been gradually increasing from the last few decades, and currently, it accounts for almost 7 million deaths per year, which is estimated to rise up to 10 million in the year 2050; with 90% of these deaths in LMICs of Africa and Asia [2, 3]. Poor policies and regulations in LMICs have increased antibiotic consumption and subsequent drug-resistant infections to a great extent [4]. Antibiotic use (ABU) in the human and animal sectors has the potential for complex One Health (OH) transmission of AMR, encompassing the environment as well. This transmission occurs through direct contact between animals and humans, and through food or shared environmental sources [5]. Moreover, intensive farming practices and disruption in environmental conditions also result in AMR transmission to both humans and animals.

Antibiotic use in food animals started in the 1940s when the tetracycline use in animals resulted in improved growth [6]. The intensive use of antibiotics in food-producing animals has increased over the last decades because of the high demand for meat [7]. Currently, antibiotics are being used in approximately 80% of food-producing animals as growth promoters [6, 8]. According to a report presented by Food and Agriculture Organization (FAO) and Organization for Economic Co-operation and Development (OECD), the estimated poultry meat production in 2014 was 108.5 million tons, while in 2023, it will reach 134.5 million tons which puts pressure on farmers to produce meat in the minimum time, e.g., 6 weeks instead of 9 or 10 weeks [9]. Undoubtedly, more antibiotic residues exist in poultry production with no or negligible withdrawal period. If the antibiotics are administered in food animals beyond the permissible limits and without adherence to the withdrawal period, this will be hazardous for human health (e.g., allergic reactions, AMR, and imbalance of intestinal microbiota) as well when they consume the meat and meat products [9]. Change in human microbiota along with the transmission of resistant genes eventually decreases the effectiveness of antibiotics used by that individual [9]. Even farmers working in the poultry production facilities may have high rates of AMR due to occupational exposure [10].

Pakistan is among the top 10 countries that are producing food animals through modern farming practices and rely on antibiotics as growth promoters and disease prevention [11]. However, there is no estimation of annual ABU in food-producing animals in Pakistan. Thus, it is difficult to estimate the exact antibiotic usage for the treatment and prevention, and as growth promoters. More than 600,000 unqualified practitioners (locally known as quacks) are active for selling these antibiotics and approx. 50,000 unregistered products are available in local markets which makes the situation even worse [12]. While Pakistan is ranked as the third highest among LMICs for antibiotic consumption [13], it is a common practice here to seek treatment from a local medical store or use antibiotics by getting advice from relatives or through previous experience. Even, several studies have reported a high percentage (50% or above) of antibiotic prescriptions at clinical setting also [14-16].

Apart from the direct effect of ABU on AMR development in humans and animals, abundance of resistant pathogens in environment and elevated environmental pressure of them are also major transmission factors in such settings. AMR transmission to the environment occurs in different ways e.g., dissemination of animal waste (faeces and urine, litter materials), grazing of animals and using organic fertilizer (animal waste), and pharmaceutical companies or human waste to the environment [17-19]. In many LMICs including rural Pakistan, poultry wastes are considered the best fertilizer for agricultural land. Antibiotics present in poultry wastes are mostly bioactive and result in increased antibiotic resistance (ABR) in exposed bacteria in the surrounding environment [20]. Therefore, the chances of resistant bacteria and gene transmission from poultry to human beings are high in rural areas because of shared living and sleeping areas with no proper waste disposal from poultry farms. Biosecurity measures are almost absent in small-scale farming in south Asia where poultry wastes are usually disposed into municipal drains or nearby open land [21].

While the burden of OH-AMR is high but difficult to quantify in LMIC settings, there are multiple challenges to mitigate it [22]. Adequate knowledge about antibiotics, optimum biosecurity and prescription practices, and AMR awareness can play a pivotal role in the rational use of antibiotics [23]. For proper policy implementation, understanding of the current poultry farming practices, the pattern of antibiotic use, and healthcare-seeking behaviour for both farmers and farm animals are crucial. Therefore, in this study, we have focused on the OH understanding of antibiotic use in commercial poultry farms and farmers along with its contributing factors in rural Pakistan.

2. Materials and Methods

2.1. Study design, study area, and recruitment

We conducted a cross-sectional OH questionnaire survey for poultry farms and poultry farmers in rural Punjab, Pakistan from January to March 2021 **(Figure 1).** We selected the Tehsil (sub-district) named Pindi Gheb from Attock district in Punjab as our study area which is one of the densely populated districts in Punjab with a large number of poultry farms. From Tehsil Pindi Gheb, out of 134 villages, we randomly selected 10

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 July 2022 doi:10.20944/preprints202207.0402.v1

villages and 4 farms per village (n=40). The eligible participants were the voluntarily agreed adult poultry farmers who provided informed consent before data collection.

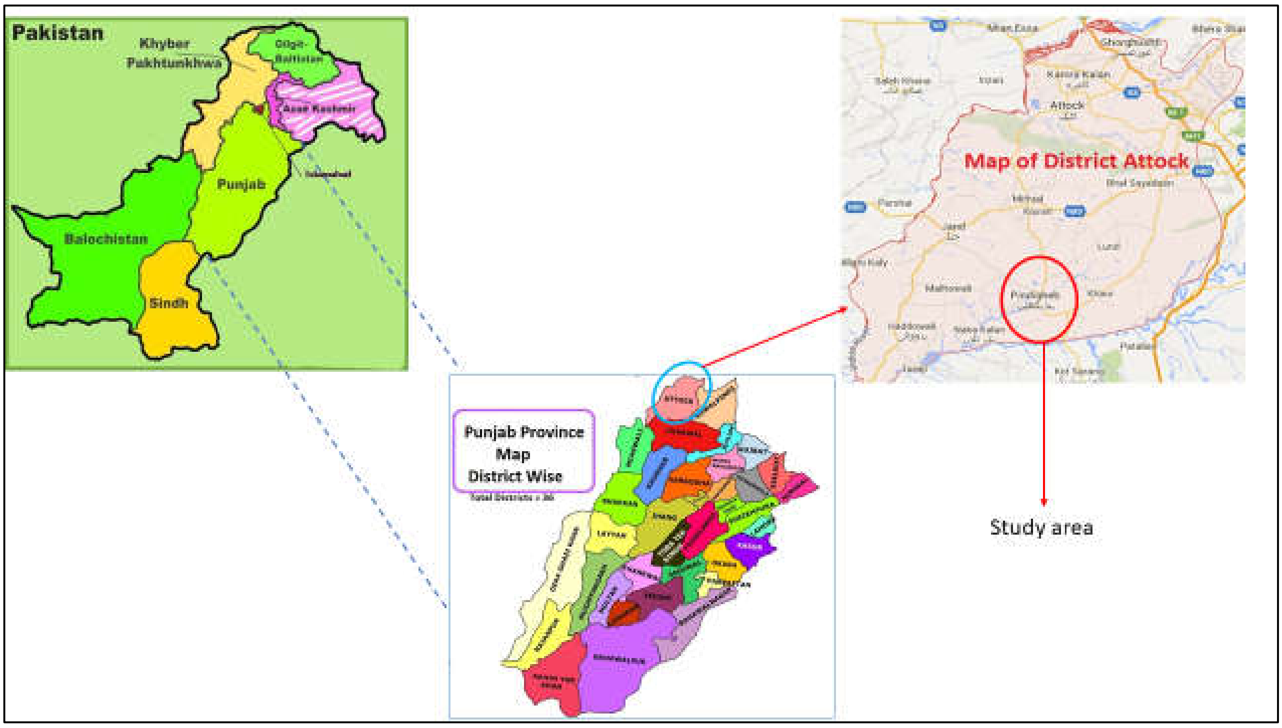

Figure 1. Map of study area [24-26].

2.2. Data collection

Data was collected using a validated and researcher administered questionnaire (pretested in 4 non-study villages). The questionnaire was translated into Urdu to make it easy for farmers to understand and all the communication with farmers was in Urdu and Punjabi (local language). We have also incorporated the suggestions and information from the local livestock officers, veterinary doctors, and medical doctors regarding the questionnaire development particularly to ask about illness and the use of antibiotics in both humans and poultry. All data were anonymized and entered in TSD (Services for sensitive data) provided by University of Oslo.

2.3. Statistical analysis

All statistical analyses were performed in IBM SPSS Statistics version 28.0.1.1 (IBM Corp.) Descriptive statistic was used to analyze the characteristics of the study farms, demographics of farmers, and pattern of antibiotic use in poultry farms and farmers. Additionally, distributions of antibiotic misuse by the demographic characteristics and the education level of farmers were compared with the knowledge of farmers about antibiotics by cross-tabulations.

We performed a chi-square test to check the association between the education level of participants and knowledge about antibiotics, ABR, and prohibited antibiotics in poultry. Statistical significance was considered at $P<0.05$.

3. Results

3.1. Demographic characteristics of the poultry farms and farmers

A total of 40 poultry rearing farms and farmers were included in the study. All farmers were male. The duration in the poultry farming profession ranged from 2 months to 35 years but nearly two-thirds of the farmers (n=25: 62.5%) had experience less than 15 years **(Table 1).**

20 farmers (50.0%) completed secondary education (10 years of education) and 10 (25.0%) had no formal education. The majority of the participants (n=35: 87.5%) never attended any professional farm training. Our enrolled poultry farms were mostly medium scale broiler farms (n=19: 47.5%) which had 2000-4000 chickens per farm **(Table 1).** The number of workers in the form also varied with the number of chickens. There was only one worker in all small-sized poultry farms having < 2000 chickens.

Table 1. Characteristics of farms and demographic data of farmers (N=40).

Characteristics	Total n (%)
No. of year/s in farming	
<15	25(62.5)
15-30	13(32.5)
>30	2(5.0)
Education level of farmers	
Not educated	10(25.0)
Primary	5(12.5)
Secondary	20(50.0)
Above Secondary	5(12.5)
Professional farm training	
No	35 (87.5)
Yes	5(12.5)
Size of Poultry farm	
Small (< 2000 chickens)	15 (37.5)
Medium (2000 to 4000 chickens)	19(47.5)
Large (>4000 chickens)	6(15.0)

3.2. Health care-seeking behaviour for antibiotic use in poultry farming

Our current study reveals the extensive use of antibiotics in all farms (n=40: 100%), major use of antibiotics as growth promoters (n=18, 45%), and lack of compliance (e.g., antibiotic administration in 50% of farms for only 1-3 days), and health care seeking from unqualified practitioners for antibiotics to a larger extent (n=24, 60%) **(Table 2).** All participants reported using antibiotics in every flock and most of them (n=33, 82.5%) reported the purchase of antibiotics from the agents instead of the pharmacy/drug stores. Agents are those who supply feed and medicines to the poultry farms and act as a third party between the poultry farmers and feed/veterinary drug companies. Moreover, 45% (n=18) of the respondents had received veterinary services from feed companies.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 July 2022 doi:10.20944/preprints202207.0402.v1

Table 2. Antibiotic use characteristics and healthcare-seeking behaviour in Poultry farming (N=40).

Variables	Total N (%)
Antibiotic/s use in poultry	
No	0(0)
Yes	40(100.0)
Veterinary doctor Prescription for getting antibiotic/s	
No	24(60.0)
Yes	16(40.0)
Source of veterinary services	
Local livestock officer	1(2.5)
Private veterinary doctor	14(35.0)
By Yourself	2(5.0)
Feed company	18(45.0)
Government source	5(12.5)
Source of getting antibiotic/s	
Agents	33(82.5)
Local pharmacy/ drug shop	7(17.5)
Use of antibiotic/s for clinical conditions	
No	18(45.0)
Yes	22(55.0)
Use of antibiotic/s as Growth promotion	
No	22(55.0)
Yes	18(45.0)
Frequency of antibiotic/s use	
Occasionally*	11(27.5)
Regularly**	29(72.5)
No. of days of antibiotic/s administration	
1-3 days	20(50.0)
4-7 days	12(30.0)
>7days	8(20,0)
Follow-up of withdrawal Period	
No	19(47.5)
Yes	21(52.5)

*Occasionally: Have not used antibiotics in every flock. **Regularly: Used antibiotics in every flock

We also found that about three-quarters of the participants (n=29, 72.5%) frequently used antibiotics. Half of the poultry farmers (n=21, 52.5%) did not follow withdrawal periods of the antibiotics. Most of the farmers (n=22, 55.0%) used antibiotics for clinical conditions, which did not require antibiotics, such as flu, fungal infections, or malaise **(Figure 2).**

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 July 2022 doi:10.20944/preprints202207.0402.v1

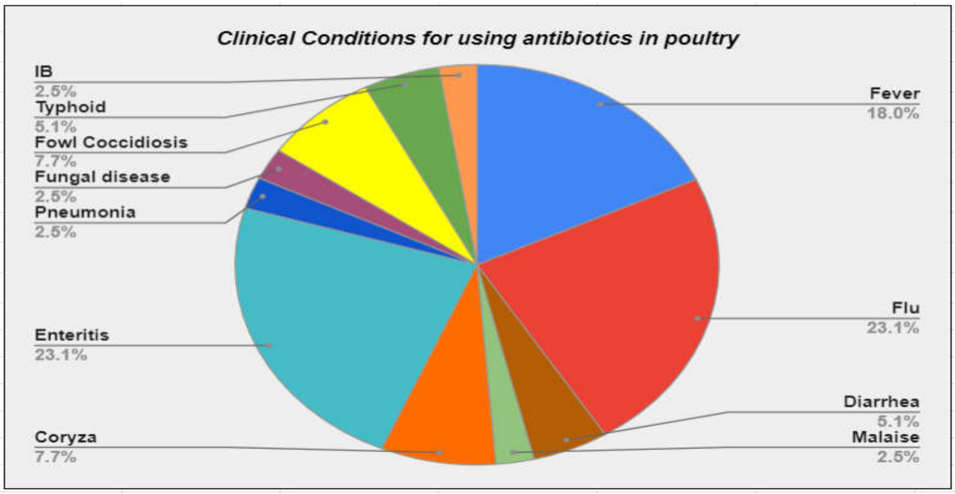

Figure 2. Clinical conditions for using antibiotics in poultry.

3.3. Pattern of antibiotic use in poultry farms and associated factors

Table 3 outlines the pattern of antibiotic use including class and types. 12 classes of antibiotics, containing 18 types, were used in poultry farming by study participants. These antibiotics were used both separately and in combination. Out of these antibiotics, both colistin and a combination of colistin sulphate and Amoxicillin trihydrate were most frequently (n=24, 60.0%) used followed by Enrofloxacin, Tylosin and Doxycycline (35.0%, 25.0% and 22.5%) respectively. Apart from antibiotics, other antimicrobials e.g., antivirals (Amantadine HCl) and antifungal (Nystatin) were used by 25.0% and 2.5% of poultry farmers for the treatment of viral and fungal diseases.

Table 3. Antibiotic classes and types used in commercial poultry farms.

Antibiotic class	Antibiotic	No of farms using antibiotics (N=40) n (%)
Aminopenicillins	Amoxicillin trihydrate	3(7.5)
Tetracyclines	Chlortetracycline	1(2.5)
	Oxytetracycline	1(2.5)
	Doxycycline	9(22.5)
Polymyxins	Colistin	24(60.0)
Macrolides	Tylosin	10(25.0)
	Erythromycin	2(5.0)
Fluoroquinolones	Ciprofloxacin	1(2.5)
	Enrofloxacin	14(35.0)
Penicillin	Penicillin	2(5.0)
Polypeptides	Bacitracin	4(10.0)
Trimethoprim	Trimethoprim	1(2.5)
Sulfonamides	Sulfamethoxypyridazine	1(2.5)
	Sulfamethazine	1(2.5)
Aminoglycosides	Neomycin	4(10.0)
	Streptomycin	2(5.0)
	Gentamycin	2(5.0)
Nitrofurans Derivatives	Furaltadone	1(2.5)
Aminopenicillins/ Polymyxins	Amoxicillin trihydrate + colistin sulphate	24(60.0)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 July 2022 doi:10.20944/preprints202207.0402.v1

As mentioned above in Table 2 that in 18 farms (45.0%) antibiotics were used as growth promoters. However, the pattern varied based on the farmers' education level, professional farm training and health seeking behaviour. As shown at **Table 4**, farmers having no education or primary level education used more antibiotics for growth promotion in poultry (n=6, 60.0% and n=4, 80.0%), as compared to those having secondary level education (n=7, 35.0%) and above (n=1, 20.0%). Likewise, professionally trained farmers had not used antibiotics as growth promoters contrary to those having no professional farm training (n=18, 51.4%). The Chi-square test indicates the significant correlation between professional farm training and antibiotic use as a growth promoter (*p= 0.05*). While education level and antibiotic use as growth promoter had no significant correlation (*p=0.141*).

Table 4. Associated factors related to use of antibiotics as growth promoters.

Farmers' characteristics n(%)	**Antibiotic/s used as growth promoter (18 out of 40)** n(%)	***p-value***
Education level of farmers 40(100.0)		*0.141*
Not educated 10(25.0	6(60.0)	
Primary 5(12.5)	4(80.0)	
Secondary 20(50.0)	7(35.0)	
Higher Secondary or graduation 5(12.5)	1(20.0)	
Professional Farm training		*0.05*
No 35 (87.5)	18(51.4)	
Yes 5(12.5)	0(0)	
Obtained antibiotics after prescription		*0.436*
No 24(60.0)	12(50.0%)	
Yes 16 (40.0)	6(37.5)	

Again, the majority (90.0%) of the respondents who were not educated had no knowledge about antibiotics use and prohibited antibiotics in poultry; and no farmer in this category had knowledge about antibiotics resistance **(Table 5).** Farmers having primary level education had no knowledge about antibiotics use, resistance, and prohibited antibiotics. Out of the 20 farmers who had secondary level education, 5 participants (25.0%) had knowledge about antibiotics use, 3 (15.0%) knew about prohibited antibiotics, and only 2 (10.0%) were knowledgeable about antibiotic use. Majority (4 out of 5, 80.0%) of the respondents having higher secondary level education or more had knowledge about antibiotic use, while over half (n=3, 60.0%) had knowledge about prohibited antibiotics and (n=2, 40.0%) had knowledge about antibiotics resistance. There is a significant association between the education level of farmers and knowledge about antibiotics use (p=0.012) and prohibited antibiotics (*p= 0.051*).

Similarly, the correlation between professional farm training and knowledge of farmers about antibiotics was statistically significant (*p<0.05*). Farmers having professional farm training (n=5) have more knowledge about antibiotics use (n=4, 80.0%) and prohibited antibiotics (n=4, 80.0%), while comparatively less knowledge about ABR (n=2, 40.0%). We have also observed that the number of years in farming has a direct relation to the knowledge about antibiotics. All farmers having more than 30 years in farming had enough knowledge, as compared to those having less experience in farming. Whereas more than 80% of the farmers had no idea about antibiotics use, ABR, and prohibited antibiotics. Therefore, these variables have statistical significance (*p<0.05*).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 July 2022 doi:10.20944/preprints202207.0402.v1

Table 5. Knowledge of poultry farmers about antibiotics.

Variables	Total N (%)	Knowledge about *AB use			Knowledge about prohibited AB			Knowledge about AB resistance		
		No N (%)	Yes N (%)	*p-value*	No N (%)	Yes N (%)	*p-value*	No N (%)	Yes N (%)	*p-value*
Education level of farmers				*0.012*			*0.051*			*0.083*
Not educated	10(25.0)	9(90.0)	1(10.0)		9(90.0)	1(10.0)		10(100)	0(0)	
Primary	5(12.5)	5(100.0)	0(0)		5(100.0)	0(0)		5(100)	0(0)	
Secondary	20(50.0)	15(75.0)	5(25.0)		17(85.0)	3(15.0)		18(90)	2(10.0)	
Higher Secondary or graduation	5(12.5)	1(20.0)	4(80.0)		2(40,0)	3(60.0)		3(60)	2(40.0)	
Professional Farm Training				*0.002*			*<0.001*			*0.017*
No	35 (87.5)	29(82.9)	6(17.1)		32(91.4)	3(8.6)		33(94.3)	2(5.7)	
Yes	5(12.5)	1(20.0)	4(80.0)		1(20.0)	4(80.0)		3(60.0)	2(40.0)	
No. of year/s in farming				*0.026*			*0.004*			*<0.001*
1-15	25(62.5)	21(84.0)	4(16.0)		23(92.0)	2(8.0)		24(96.0)	1(4.0)	
15-30	13(32.5)	9(69.2)	4(30.8)		10(76.9)	3(23.1)		12(92.3)	1(7.7)	
>30	2(5)	0(0)	2(100.0)		0(0)	2(100.0)		0(0)	2(100.0)	

3.4. Environmental dissemination of poultry wastes

To identify the environmental dissemination of AMR from poultry farming, we collected information about waste disposal practices. Most of the poultry farmers (85.0%) reported not having any wastewater drainage system in their farms; rather the poultry waste was being directly drained into the adjacent open areas and agricultural land. Only 6 farms (15.0%) had proper drainage systems. Additionally, 24 (60.0%) farmers have reported that they use poultry wastes as fertilizer, which is causing the further spread of AMR to the food system.

3.5. Health care-seeking behaviour and antibiotic use in humans (poultry farmers)

Out of the 40 participants, more than one-third (n=15, 37.5%) used antibiotics within the last month preceding the survey, (n=2, 5.0%) in the last 1-3 months, while (n=10, 25.0%) of the participants used antibiotics 6 months prior to the survey. Two-thirds (n=13, 32.5%) of the participants did not remember the last intake of antibiotics. About half (n= 21, 52.5%) of the respondents reported self-medication of antibiotics without a physician's prescription. Almost half of the participants (n=19, 47.5%) took previously used antibiotics without consulting a physician, while (n=1, 2.5%) used antibiotics after getting advice from relatives. One participant (2.5%) mentioned that he had no access to physicians, so he used antibiotics without prescription.

When participants were asked about the source of antibiotics, (n=30,75.0%) reported to obtaining them from local pharmacies, (n=8, 20.0%) from leftover antibiotics at home, and (n=2, 5.0%) obtained from rural medical practitioners (unqualified doctors). Moreover (n=28,70.0%) of the respondents used antibiotics for 1-3 days, (n=8, 20.0%) used for 4-7 days, and (n=4, 10.0%) used for more than 7 days. **(Table 6)**

doi:10.20944/preprints202207.0402.v1

Table 6. Pattern of antibiotics use in poultry farmers (N=40).

Characteristics	Total n (%)
Purpose of antibiotic/s use	
Flu (common cold)	17(42.5)
Gastrointestinal infections	4(10.0)
Respiratory infections	13(32.5)
Fever	3(7.5)
Others*	3(7.5)
Physician prescription	
No	21(52.5)
Yes	19(47.5)
Reason behind self-medication	
None	19(47.5)
Not access to physician care	1(2.5)
Previous experience	19(47.5)
Advice from relatives	1(2.5)
Source of getting antibiotic/s	
Pharmacy	30(75.0)
Leftover household antibiotics	8(20.0)
Rural practitioner (Untrained doctor)	2(5.0)
Duration of antibiotic/s use	
1-3 days	28(70.0)
4-7 days	8(20.0)
>7 days	4(10.0)

*Others include skin infections (n=2) and Inguinal hernia (n=1).

Considering the indications of ABU, a large proportion (n=17, 42.5%) of the participants mentioned treating flu/common cold (mostly viral), and about one-third (n= 13, 30.0%) stated respiratory infections in general (where cough and chest pain were common symptoms) **(Figure 3).**

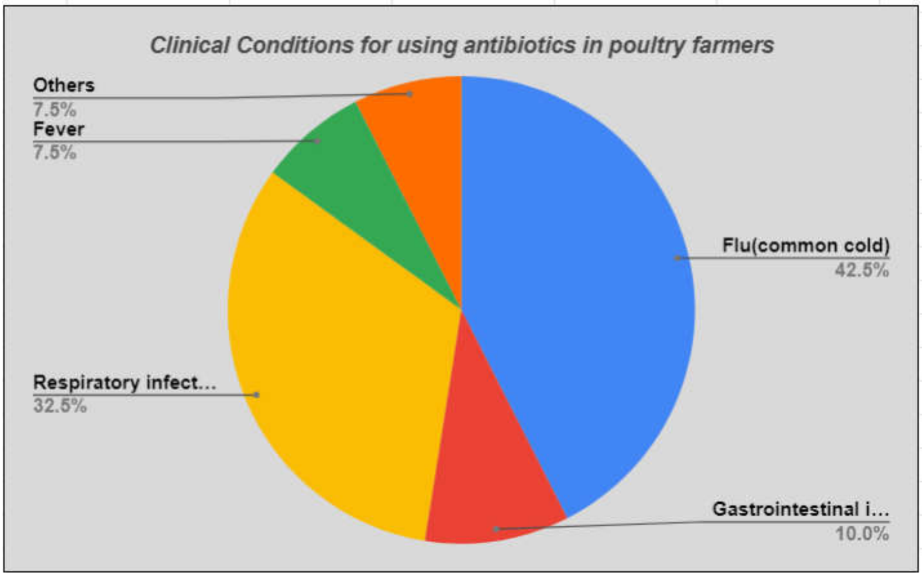

Figure 3. Clinical Conditions for using antibiotics in poultry farmers.

 doi:10.20944/preprints202207.0402.v1

4. Discussion

In the current study, we have evaluated the practice of antibiotic use and healthcare-seeking behaviour regarding the poultry farming and farmers in rural areas of Punjab, Pakistan in OH approach which is the first of this nature in the study area.

Our study findings confirm that the use of antibiotics in poultry is not well regulated in Pakistan, particularly in rural areas and it has the potential to enhance the emergence of drug resistant pathogens to develop AMR. Most of the participants used antibiotics as growth promoters without any consultation with trained veterinarians. This observation of the unregulated use of antibiotics in food-producing animals in Pakistan including improper dosage, wrong combination of antibiotics, misuse, and overuse is similar to other studies in similar settings [11, 27, 28]. In addition, we observed a significant seasonal variation in prophylactic antibiotic use in poultry. The poultry farmers use more antibiotics in winter than summer as chickens are more prone to diseases in cold weather. This information combined with antibiotic sale report at different time points can be crucial for policy implementation.

The majority of the participants in this study purchased antibiotics based on their previous experience and from local agents, which is a clear indication of patron-client relationship and undue influence for unnecessary usage. Such resistance-provoking drug purchase behaviour and practice are also evident in similar LMIC settings [11, 29-31]. Our study participants mostly used colistin sulphate and amoxicillin trihydrate, which is alarming. Overuse and misuse of colistin lead to the development of multidrug resistance as reported in previous studies [32-34]. Some farmers used antibiotics as a supplement on daily basis when few used antibiotics on alternative days without following the duration of treatment and withdrawal time. Another important finding was the inability of the participants to distinguish between viral and bacterial infections which supports the fact that nearly half of them used antibiotics for flu (common cold) and a few used for fever, supported by other studies done in Punjab, Pakistan [35, 36]. Lack of education, lack of professional farm training, and not getting advice from the veterinary doctor were the common reasons behind such misuse and these findings are in line with previous studies [11, 30, 37].

Another important concern of antibiotic use in poultry farming is the 'Withdrawal period'. Any medicine or antibiotic consumed by humans or animals, has a withdrawal period when they become non-functional and eliminated from the body. The 'Withdrawal period' is particularly important for food animals such as poultry, cattle to ensure that no antibiotics have entered the human food chain. Unfortunately, nearly half of our participants were unaware of this term and so did not follow the recommended withdrawal period. This unhealthy practice increases the possibility of high level of antibiotic residues in poultry meat with detrimental health consequences. All these findings of violation of the withdrawal period for antibiotics have also been observed in other studies [29, 31, 38].

Antibiotic use in poultry and lack of proper biosecurity practices are major concerns in the environmental dissemination of antibiotic residue and resistant bacteria which in turn act as the mixing hub of human-animal superbugs. A majority of farms in the current study had no wastewater drainage system, and they drained wastewater into nearby agricultural land or open sites near farms. This practice increases the chances of antibiotic contamination of agricultural land through raw and treated wastewater [17]. Moreover, farmers sold poultry wastes to agricultural landowners to be used as fertilizer and even, more than half of the participants utilized poultry wastes as fertilizers by themselves as well. Several previous studies have revealed the linkage between antibiotic use in poultry and the development of AMR in humans and in surroundings through antibiotic residue in manure and urine [39-41].

In terms of ABU among poultry farmers in the study area, the easy accessibility of antibiotics from pharmacies/drug stores without doctors' prescriptions is an important issue. One-third of the participants obtained antibiotics from pharmacies and self-medication is a common practice. People in LMIC settings have no idea about the risk of self-medication and they purchase antibiotics from the drug store without physicians' prescription [13, 42, 43]. Another concern is the use of antibiotics from previous experience and from leftover at home. Our study participants also reported this practice. The main reason behind this was the financial constraints and traveling to the cities to seek physician's consultation. This observation has also been reported in studies conducted in India, Sudan, and Nigeria [44-46].

Several studies have reported that patients understanding about illness and treatment will increase their adherence to the medication [47, 48]. In our findings, the drug adherence to antibiotics was not proper and most of the participants used antibiotics for 1-3 days. Participants were of the opinion that they stop taking medicine after they feel better. Improper consumption of antibiotics results in antibiotic resistance [49]. Incomplete information about antibiotic use, getting only a few doses because of high prices, and use of left-over antibiotics at home are the reasons associated with it [13]. Even from pharmacies or from rural practitioners, one can get antibiotics for one-day treatment. However, non-adherence to antibiotic regimen can be improved by increasing general population knowledge and proper counselling at pharmacies and by improving pharmacist-patient interactions [49].

Knowledge about ABR and antibiotics use is a fundamental requirement to mitigate AMR at community level. A significant number of our study participants had no knowledge about these issues. Knowledge of the farmers about antibiotics was directly associated with their education level. Uneducated participants and those with primary level education had no or limited knowledge about antibiotic use, ABR, and prohibited list of antibiotics in poultry as compared to those participants who had secondary or more education, and these findings are in consistent with other studies [13, 50, 51]. Therefore, educational interventions can be effective to raise awareness, enhance knowledge about antibiotic use, and changing their healthcare-seeking behaviour. A good example is E-bug by public health England which is an international health education source to aware and educate people about antibiotics, ABR, and infections [52, 53].

While our study focuses on an imperative aspect of OH-AMR in rural Pakistan, it has few limitations. The findings may not be generalized to the whole country as we collected data from a sub-district in Punjab. Yet, these results provide a descriptive picture of the overall situation of antibiotic use in rural Pakistan. Moreover, the findings of this study may also be affected by recall bias to some extent as participants had to remember the use of antibiotics and they have very minimal medicine-related knowledge. However, we tried to validate the findings by collecting and inspecting the antibiotic boxes from the farms and households.

5. Conclusion

Our study highlights the risks of AMR due to non-professional farming practices and its hazards to humans, animals, and the environment. It asserts the need for education and professional farm training for the containment of AMR in resource-deficient settings. The current study also contends the alignment of food safety policy with the current AMR mitigation plan. An integrated and sustainable national AMR and food safety policy needs to be adopted with the inclusion of farmers' education, mass awareness, organic farming, and strict antibiotic usage guidelines.

Author Contributions: Conceptualization- M.A.; Methodology- M.A. and U.E.H.; Formal Analysis-U.E.H. and M.A.; Resources-M.A. and A.K.; Data Curation- U.E.H.; Writing – Original Draft Preparation- M.A. and U.E.H.; Writing – Review & Editing- E.J.M. and A.K.; Supervision-M.A., A.K. and E.J.M.; Project Administration- M.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Department of Community Medicine and Global Health, Institute of Health and Society, Faculty of Medicine, University of Oslo, Norway.

Institutional Review Board Statement: Ethical approval for this study was obtained from the Ethics Committee at Institute of health and society, UiO (Application no. 8849626), Norwegian Centre for Research Data (reference no. 726029) and the Bioethics Committee of faculty of biological sciences, Quaid-i-Azam University, Islamabad, Pakistan (reference no. #BEC-FBS-QAU2021-250).

Informed Consent Statement: All data was anonymised prior to analysis and publication. A written informed consent was obtained from each participant before participation in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Nayiga, S., et al., *Use of antibiotics to treat humans and animals in Uganda: a cross-sectional survey of households and farmers in rural, urban and peri-urban settings.* JAC-Antimicrobial Resistance, 2020. **2**(4): p. dlaa082.
2. de Kraker, M.E., A.J. Stewardson, and S. Harbarth, *Will 10 million people die a year due to antimicrobial resistance by 2050?* PLoS medicine, 2016. **13**(11): p. e1002184.
3. Hofer, U., *The cost of antimicrobial resistance.* Nature Reviews Microbiology, 2019. **17**(1): p. 3-3.
4. Van Boeckel, T.P., et al., *Global trends in antimicrobial resistance in animals in low-and middle-income countries.* Science, 2019. **365**(6459): p. eaaw1944.
5. Landers, T.F., et al., *A review of antibiotic use in food animals: perspective, policy, and potential.* Public health reports, 2012. **127**(1): p. 4-22.
6. Zellweger, R.M., et al., *A current perspective on antimicrobial resistance in Southeast Asia.* Journal of Antimicrobial Chemotherapy, 2017. **72**(11): p. 2963-2972.
7. Collignon, P. and A. Voss, *China, what antibiotics and what volumes are used in food production animals?* 2015, BioMed Central. p. 1-4.
8. Darwish, W.S., et al., *Antibiotic residues in food: the African scenario.* Japanese Journal of Veterinary Research, 2013. **61**(Supplement): p. S13-S22.
9. Muaz, K., et al., *Antibiotic residues in chicken meat: global prevalence, threats, and decontamination strategies: a review.* Journal of food protection, 2018. **81**(4): p. 619-627.
10. Braykov, N.P., et al., *Antibiotic resistance in animal and environmental samples associated with small-scale poultry farming in northwestern Ecuador.* Msphere, 2016. **1**(1): p. e00021-15.
11. Ur Rahman, S. and M. Mohsin, *The under reported issue of antibiotic-resistance in food-producing animals in Pakistan.* Pak. Vet. J, 2019. **1**: p. 1-16.
12. Rahman, S.U., S. Ahmad, and I. Khan, *Incidence of ESBL-producing-Escherichia coli in poultry farm environment and retail poultry meat.* Pak Vet J, 2018. **39**: p. 116-20.
13. Atif, M., et al., *What drives inappropriate use of antibiotics? A mixed methods study from Bahawalpur, Pakistan.* Infection and drug resistance, 2019. **12**: p. 687.
14. Atif, M., et al., *Assessment of core drug use indicators using WHO/INRUD methodology at primary healthcare centers in Bahawalpur, Pakistan.* BMC health services research, 2016. **16**(1): p. 1-9.
15. Atif, M., et al., *Assessment of WHO/INRUD core drug use indicators in two tertiary care hospitals of Bahawalpur, Punjab, Pakistan.* Journal of pharmaceutical policy and practice, 2016. **9**(1): p. 1-8.
16. Hafeez, A., et al., *Prescription and dispensing practices in public sector health facilities in Pakistan-survey report.* Journal-Pakistan Medical Association, 2004. **54**(4): p. 187-191.
17. Tasho, R.P. and J.Y. Cho, *Veterinary antibiotics in animal waste, its distribution in soil and uptake by plants: A review.* Science of the Total Environment, 2016. **563**: p. 366-376.
18. Kumar, K., et al., *Antibiotic uptake by plants from soil fertilized with animal manure.* Journal of environmental quality, 2005. **34**(6): p. 2082-2085.
19. Elmund, G.K., et al., *Role of excreted chlortetracycline in modifying the decomposition process in feedlot waste.* Bulletin of Environmental Contamination and Toxicology, 1971. **6**(2): p. 129-132.
20. Barnes, K.K., et al., *A national reconnaissance of pharmaceuticals and other organic wastewater contaminants in the United States—I) Groundwater.* Science of the total environment, 2008. **402**(2-3): p. 192-200.
21. Rousham, E.K., L. Unicomb, and M.A. Islam, *Human, animal and environmental contributors to antibiotic resistance in low-resource settings: integrating behavioural, epidemiological and One Health approaches.* Proceedings of the Royal Society B: Biological Sciences, 2018. **285**(1876): p. 20180332.
22. Sartelli, M., et al., *Antibiotic use in low and middle-income countries and the challenges of antimicrobial resistance in surgery.* Antibiotics, 2020. **9**(8): p. 497.

23. Napolitano, F., et al., *The knowledge, attitudes, and practices of community pharmacists in their approach to antibiotic use: A nationwide survey in Italy.* Antibiotics, 2019. **8**(4): p. 177.
24. *TRIBES OF ATTOCK DISTRICT*. 2014 [cited 2022; Available from: https://newpakhistorian.wordpress.com/tag/tribes-of-attock-district/.
25. *Punjab Province – List of Tehsils, Districts and Divisions*. 2017 [cited 2022; Available from: https://www.politicpk.com/punjab-province-list-tehsils-districts-divisions-%D8%B5%D9%88%D8%A8%DB%81-%D9%BE%D9%86%D8%AC%D8%A7%D8%A8/.
26. *Administrative divisions map of Pakistan*. [cited 2022; Available from: https://ontheworldmap.com/pakistan/administrative-divisions-map-of-pakistan.html.
27. Annan-Prah, A., et al., *Antibiotic use, abuse and their public health implication: the contributory role of management flaws in the poultry industry in two agro-ecological zones in Ghana.* 2012.
28. Nisar, M., et al., *Prevalence and antimicrobial resistance patterns of Campylobacter spp. isolated from retail meat in Lahore, Pakistan.* Food control, 2017. **80**: p. 327-332.
29. Al-Mustapha, A.I., V.O. Adetunji, and A. Heikinheimo, *Risk perceptions of antibiotic usage and resistance: A cross-sectional survey of poultry farmers in Kwara State, Nigeria.* Antibiotics, 2020. **9**(7): p. 378.
30. Glasgow, L., et al., *Antibiotic use in poultry production in Grenada.* Veterinary medicine international, 2019. **2019**.
31. Guetiya Wadoum, R., et al., *Abusive use of antibiotics in poultry farming in Cameroon and the public health implications.* British poultry science, 2016. **57**(4): p. 483-493.
32. Agency, E.M., *Use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health*. 2013, European Medicines Agency London, UK.
33. Organization, W.H., *Global Antimicrobial Resistance Surveillance System (GLASS): the detection and reporting of colistin resistance*. 2018, World Health Organization.
34. Wongsuvan, G., et al., *Antibiotic use in poultry: a survey of eight farms in Thailand.* Bulletin of the World Health Organization, 2018. **96**(2): p. 94.
35. Arshad, M.S., M. Ijaz, and A. Hussain, *Evaluation of antibiotic use behavior in cold and flu amongst the students of Bahauddin Zakariya University Multan Pakistan.* Pak J Pharm, 2010. **23**(2): p. 15-22.
36. Anwar, M., et al., *Self-medication, home remedies, and spiritual healing: common responses to everyday symptoms in Pakistan.* Health Psychology and Behavioral Medicine, 2015. **3**(1): p. 281-295.
37. Xu, J., et al., *Antibiotic use in chicken farms in northwestern China.* Antimicrobial Resistance & Infection Control, 2020. **9**(1): p. 1-9.
38. Turkson, P., *Use of drugs and antibiotics in poultry production in Ghana.* Ghana Journal of Agricultural Science, 2008. **41**(1).
39. Heuer, H. and K. Smalla, *Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months.* Environmental microbiology, 2007. **9**(3): p. 657-666.
40. Kelley, T.R., et al., *Antibiotic resistance of bacterial litter isolates.* Poultry Science, 1998. **77**(2): p. 243-247.
41. Yeom, J.-R., S.-U. Yoon, and C.-G. Kim, *Quantification of residual antibiotics in cow manure being spread over agricultural land and assessment of their behavioral effects on antibiotic resistant bacteria.* Chemosphere, 2017. **182**: p. 771-780.
42. Shet, A., S. Sundaresan, and B.C. Forsberg, *Pharmacy-based dispensing of antimicrobial agents without prescription in India: appropriateness and cost burden in the private sector.* Antimicrobial resistance and infection control, 2015. **4**(1): p. 1-7.
43. Zapata-Cachafeiro, M., et al., *Determinants of antibiotic dispensing without a medical prescription: a cross-sectional study in the north of Spain.* Journal of Antimicrobial Chemotherapy, 2014. **69**(11): p. 3156-3160.
44. Banerjee, I. and T. Bhadury, *Self-medication practice among undergraduate medical students in a tertiary care medical college, West Bengal.* Journal of postgraduate medicine, 2012. **58**(2): p. 127.
45. Omolase, C., et al., *Self medication amongst general outpatients in a Nigerian community hospital.* Annals of Ibadan postgraduate medicine, 2007. **5**(2): p. 64-67.
46. Salim, A.M. and B. Elgizoli, *Exploring the reasons why pharmacists dispense antibiotics without prescriptions in Khartoum state, Sudan.* International Journal of Pharmacy Practice, 2017. **25**(1): p. 59-65.
47. Kincey, J., P. Bradshaw, and P. Ley, *Patients' satisfaction and reported acceptance of advice in general practice.* The Journal of the Royal College of General Practitioners, 1975. **25**(157): p. 558-566.
48. Sharpe, T.R. and R.L. Mikeal, *Patient compliance with antibiotic regimens.* American Journal of Health-System Pharmacy, 1974. **31**(5): p. 479-484.
49. West, L.M. and M. Cordina, *Educational intervention to enhance adherence to short-term use of antibiotics.* Research in Social and Administrative Pharmacy, 2019. **15**(2): p. 193-201.
50. Napolitano, F., et al., *Public knowledge, attitudes, and experience regarding the use of antibiotics in Italy.* PloS one, 2013. **8**(12): p. e84177.
51. Nazir, S. and M. Azim, *Assessment of antibiotic self-medication practice among public in the northwestern region of Pakistan.* European Journal of Hospital Pharmacy, 2017. **24**(4): p. 200-203.
52. Eley, C.V., et al., *Young people's knowledge of antibiotics and vaccinations and increasing this knowledge through gaming: mixed-methods study using e-Bug.* JMIR Serious Games, 2019. **7**(1): p. e10915.
53. Tsuzuki, S., et al., *Factors associated with sufficient knowledge of antibiotics and antimicrobial resistance in the Japanese general population.* Scientific reports, 2020. **10**(1): p. 1-9.