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INVESTIGATING ANTIMICROBIAL RESISTANCE PRACTICES IN PAKISTAN'S LIVESTOCK SECTOR: IMPLICATIONS FOR HUMAN AND ANIMAL HEALTH POLICY

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Abstract

Antimicrobial resistance (AMR) poses an increasing threat to global public health, particularly in low- and middle-income nations where regulatory control over antibiotic utilization is inadequate. The unregulated and excessive use of antimicrobials in Pakistan's poultry and dairy cattle sectors has led to the development of drug-resistant bacterial strains, posing a threat to both animal and human health. This study examined the incidence of antimicrobial resistance (AMR) and its behavioral determinants in a veterinary context, utilizing a One Health approach to evaluate hazards at the animal-human interface. This study aimed to assess antimicrobial resistance patterns in significant pathogens and investigate the knowledge, attitudes, and practices regarding antibiotic use among stakeholders in Pakistan's poultry and dairy cow industries. A cross-sectional, mixed-methods study was conducted at University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan. Data was gathered from 300 livestock stakeholders via structured surveys, and 100 biological samples were examined for resistant strains of Escherichia coli, Salmonella spp., and Staphylococcus aureus using the Kirby-Bauer disk diffusion method. Statistical methods, including chi-square tests and regression models, were employed to assess the relationship between antibiotic use and the development of resistance. The findings indicated significant resistance levels among all three infections, with S. aureus in dairy cattle exhibiting the highest resistance at 88% and E. coli in poultry also displaying considerable rates at 87%. Survey findings revealed that 68% of respondents used antibiotics without veterinary prescriptions, and more

than half were unaware of antimicrobial resistance (AMR). A substantial correlation was identified between inadequate awareness and antibiotic abuse (P < 0.05). These findings underscore the pressing need for targeted awareness initiatives, veterinary management programs, and policy measures to mitigate the irrational use of antibiotics. This study offers essential evidence to endorse integrated antimicrobial resistance control strategies in Pakistan's animal health systems.

INTRODUCTION

AMR is one of the greatest public health threats of the 21st century, resulting in significant societal and economic consequences in both human and veterinary medicine (Fuller et al., 2022). AMR is the acquired ability of microbes to survive the intended effect of antibiotic treatments that were previously effective for the prevention and treatment of infections, causing longer illnesses, more deaths, and higher treatment costs (O'Neill, 2016). In low- and middle-income countries, the substandard sale of antibiotics exacerbates the situation, but the cause is not only the availability of antibiotics; there is also a lack of awareness about resistance data and veterinary inspections (Laxminarayan et al., 2013). The livestock production chain, particularly poultry and dairy farms, is a pivotal point of overlap in the emergence and spread of antimicrobial resistance (AMR) resulting from the misuse of antibiotics for disease prevention, growth stimulation, and treatments (Van Boeckel et al., 2015). The overuse and misuse of antimicrobials in food animals play a significant role in the emergence of resistant organisms that can be transmitted to humans through direct contact or the consumption of contaminated products (Tang et al., 2017). Pakistan is one of the leading livestock-producing countries, and the abuse of antibiotics in its livestock sector is a significant public health risk that must be addressed promptly and through informed decisions. However, most of the prevalent policy discourse on AMR in the region has centered disproportionately on human health, with little emphasis on veterinary aspects (Abdullah et al., 2025). Multiple studies have highlighted the prevalence of drug-resistant bacteria in envi-ronments where livestock are maintained and the critical necessity of curtailing the misuse of antibiotics in animal agriculture. For example, Umair et al. (2024) reported resistance to fluoroquinolones and aminoglycosides among E. coli isolates recovered from poultry farms in Punjab, indicating the excessive use of critically important antimicrobials in broiler production. Similarly, Jamali et al. (2014) observed an elevated resistance of S. aureus to penicillin in cases of mastitis in dairy cattle. They associated the lack of an appropriate treatment program with a growing number of therapeutic failures. Such studies are important for understanding the pathogen-specific resistance profile and its impact on food safety. However, Ahmed et al. (2023) noted that there is a paucity of combined research investigating antimicrobial use patterns, knowledge levels, and resistance outcomes across animal and human medicine, incorporating both sectors to provide a cohesive One Health perspective. In addition, previous studies were often confined to a specific geographical area, conducted in laboratory settings, or focused specifically on human or animal health, lacking an overall view of antimicrobial resistance (AMR) in the community. These literature gaps argue for the use of integrated and cross-sectional studies to reveal the interconnected factors associated with AMR within high-risk settings, such as the livestock sector in Pakistan.

This study aimed to investigate the prevalence of antibiotic resistance in pathogens isolated from poultry and dairy cattle and to assess the role of knowledge, attitudes, and practices of stakeholders involved in the use of antibiotics and animal healthcare provision. In contrast to previous studies, this study introduces a cross-sectional One Health perspective that is original in that it collects both microbiological and behavioral data. As Ahmad et al. (2023) highlighted, linking laboratory diagnostics with field-level behavioral analysis provides a more nuanced perspective on AMR pathways and permits more targeted policy interventions. This methodological innovation is based on the premise that AMR is not just a biomedical problem but also a

socio-behavioral one, encompassing knowledge gaps, regulatory voids, and market practices. This study is based on the fundamental work of Malik et al. (2020), who underscored the importance of interdisciplinary strategies in tackling antimicrobial resistance (AMR) in livestock production systems of South Asia. Building upon this body of work, the current study addresses significant limitations of previous research by investigating resistance across multiple provinces, examining a diverse array of livestock systems, and employing a mixed-methods approach. This approach incorporates both empirical data on resistance and the human aspects of antibiotic usage. Consequently, this contributes to the essential evidence base for context-specific antimicrobial resistance (AMR) intervention programming in Pakistan and other regions.

1. METHODOLOGY

2.1 STUDY DESIGN AND APPROACH

This study design was employed to investigate the relationship between antimicrobial utilization and the emergence of resistance in veterinary and human health within the Pakistani livestock sector conducted at University of Veterinary and Animal Sciences, Lahore. After consulting with an expert panel, it was determined that a cross-sectional design was suitable for collecting data on a broad cross-section of the population at a single point in time. This design aligns well with the study's objectives, which aim to gather knowledge about current behavior, treatment use, and resistance prevalence among key groups, including livestock farmers, veterinarians, and human healthcare professionals. Furthermore, the cross-sectional design is most applicable to the association of antibiotic abuse with pathogen resistance in a resource-constrained country such as Pakistan.

2.2 DATA COLLECTION METHODS

This cross-sectional, mixed-methods study employed structured surveys and microbiological laboratory testing. Structured questionnaires were formulated and provided to livestock owners, veterinarians, and human health practitioners to collect information regarding the patterns of antibiotic use, knowledge, and hygiene behavior. Expert input was utilized to develop and refine these surveys, ensuring they were precise, reliable, and content-valid in relation to the research objectives.

Concurrently, poultry and dairy cattle samples were collected to detect the presence of AMR pathogens and were also studied. The samples consisted of feces, mastitis cow milk, and poultry meat swabs. In vitro, disc diffusion, and minimum inhibitory concentration (MIC) methods were employed for laboratory analysis based on internationally established CLSI recommendations. Participants and livestock sites were selected using stratified random sampling. The sample was stratified by region (Punjab, Sindh, Khyber Pakhtunkhwa) and type of livestock (poultry, dairy cows) to ensure a broader representation of the sample. The sample size was established to include 300 individuals and 100 biological samples for testing purposes.

2.3 VARIABLES AND MEASURES

Both the behavioral and microbiological dimensions of antimicrobial resistance were considered in the study through a set of independent and dependent variables. The explanatory variables were the type and frequency of antibiotics administered, availability of consultation with a veterinarian, and biosecurity on farms/clinics. These were measured using Likert-scale items and types of responses on the questionnaire.

The outcome variables were the prevalence of resistant strains of Escherichia coli, Salmonella spp., and Staphylococcus aureus in livestock samples and cases of reported treatment failure and observed zoonotic symptoms in humans. The presence and level of resistance were defined using laboratory methods, such as the Kirby-Bauer disc diffusion test and MIC determination, which allowed an unbiased quantification of AMR. These measures are consistent with the hypothesized relationships and aims of the study and support a strong analytic examination of practice-outcome correlations.

2.4 SAMPLING TECHNIQUE

Stratified random sampling was employed to enhance the generalizability of the data to the broader livestock and human populations. The study population was stratified by livestock type (poultry and dairy cattle) and geo-stratification (Punjab, Sindh, and KPK). Farmers, veterinarians, and general healthcare facilities were randomly selected from each stratum. The participation criteria were the experience of at least one year in livestock upkeeping or livestock

disease management. This sampling technique was chosen to enhance generalizability and consider the diversity in the structure of the agricultural and healthcare industries in Pakistan.

2.5 DATA ANALYSIS PLAN

The gathered data were statistically analyzed using both descriptive and inferential statistics, and the results were represented using SPSS (Version 26) and Microsoft Excel. Demographics, frequencies, means, and standard deviations were used to describe antibiotic utilization practices and knowledge levels. The chi-square test and binary logistic regression model were used to assess the statistical relationship between the independent variable (type of antibiotic) and the dependent variable (resistance type). Resistance rates were obtained and compared among regions and between livestock groups using analysis of variance (ANOVA) to determine the antimicrobial concentration of resistance (ACOR) in laboratory diagnosis.

2. RESULTS

This study evaluated antimicrobial resistance (AMR) patterns in *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus* isolated from poultry and dairy cattle in three provinces of Pakistan. It also assessed the knowledge, attitudes, and practices of stakeholders regarding antibiotic usage.

3.1 PREVALENCE AND RESISTANCE PATTERNS OF PATHOGENS

Laboratory analysis of 100 biological samples revealed high levels of antimicrobial resistance across both poultry and dairy cattle samples. *Staphylococcus aureus* emerged as the most resistant pathogen in dairy cattle. The observed resistance levels for each pathogen by livestock category are presented in **Table 3.1**.

Table 3.1 Antimicrobial Resistance (%) in Poultry and Dairy Cattle Isolates

Pathogen	Poultry Resistance (%)	Dairy Cattle Resistance (%)
E. coli	87%	86%
Salmonella spp.	78%	72%
Staphylococcus aureus	84%	88%

As indicated in Table 3.1, resistance to commonly used antibiotics is alarmingly high in both livestock types. This pattern is further illustrated in **Figure 3.1**, where the resistance rates for each pathogen are compared side-by-side.

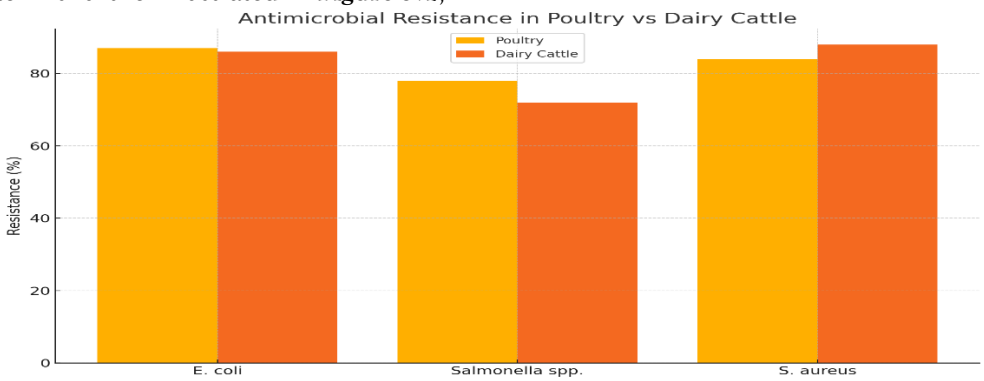

Figure 3.1: This bar chart compares resistance rates of E. coli, Salmonella spp., and S. aureus in poultry and dairy cattle. S. aureus in dairy cattle shows the highest resistance, indicating overuse of antibiotics in mastitis treatment protocols.

3.2 SURVEY-BASED ANALYSIS OF ANTIBIOTIC PRACTICES

From the 300 stakeholders surveyed, 92% of poultry farmers and 87% of dairy cattle handlers reported recent antibiotic use. A significant 68% admitted to purchasing antibiotics without prescriptions.

Oxytetracycline, colistin, and penicillin were among the most used drugs.

Survey responses also revealed a gap in AMR awareness and training. Only 45% of respondents were aware of the concept of antimicrobial resistance, and just 28% had received any training on rational antibiotic use. These findings are visually summarized in **Figure 3.2**.

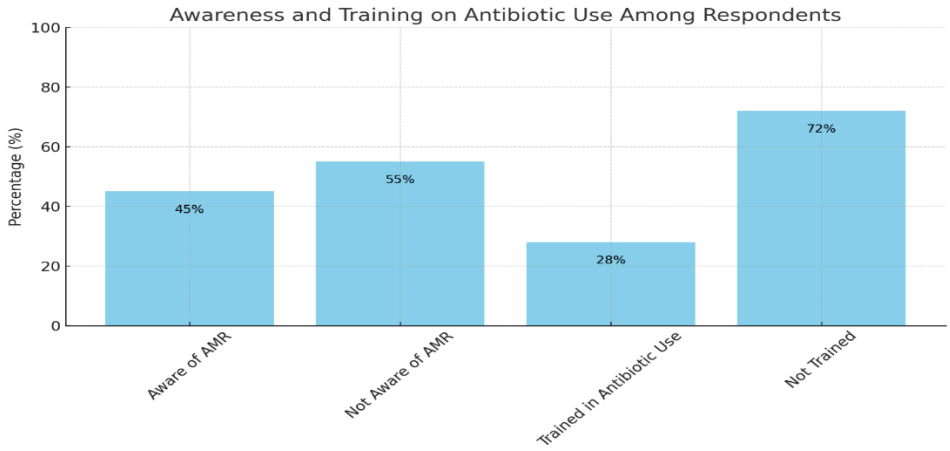

Figure 3.2: This chart shows the percentage of participants aware of AMR and those trained in antibiotic usage. A large majority lacked formal training or awareness, highlighting critical gaps in AMR education in the livestock sector.

A chi-square analysis demonstrated a statistically significant correlation ($p < 0.05$) between lack of awareness and inappropriate antibiotic use, including non-prescription purchases and incorrect dosages.

3.3 GEOGRAPHIC AND OPERATIONAL TRENDS

Regional analysis revealed Punjab to have the highest incidence of AMR, followed by Sindh and Khyber Pakhtunkhwa. Resistance levels were significantly higher in commercial poultry operations in Punjab, particularly for *E. coli* and *Salmonella* spp. In contrast, dairy operations in Sindh and KP exhibited slightly lower resistance rates, though *S. aureus* resistance remained critically high in cattle affected by mastitis.

3. DISCUSSION

The data reported in this study are essential for understanding antimicrobial resistance (AMR) associated with poultry and dairy cattle in the livestock sector in Pakistan. The results of this study showed high resistance in bacterial isolates from E. coli, Salmonella spp., and Staphylococcus aureus, with S. aureus from dairy cattle exhibiting the highest resistance at 88% (Haq et al., 2024). The survey findings revealed disturbing rates of misuse of antibiotics and access without prescriptions, as well as a poor understanding of AMR by animal handlers. Only 45% of the participants had heard of AMR, and only 28% had received training on the subject (Ahmed et al., 2023).

The high resistance of Staphylococcus aureus from dairy cows suggests the use of nonspecific antibiotics in mastitis therapy, likely without sensitivity testing based on culture results (ALI, 2020). In poultry, the high resistance of E. coli and Salmonella indicates that prophylactic antibiotics and growth promoters are commonly used (Umair et al., 2024). This strong association between the absence of AMR education and inappropriate antibiotic use supports the involvement of behavioral and awareness gaps that have contributed to the resistance crisis (Laxminarayan et al., 2013). These results are consistent with the hypothesis that ineffective antibiotic stewardship, insufficient veterinary oversight, and lax enforcement of regulations contribute to the increasing burden of AMR in the livestock sector in Pakistan.

These results are consistent with those of other studies conducted in Pakistan and other income countries. For instance, Ahmed et al. (2023) described analogous patterns of resistance in poultry isolates, including a high proportion of tetracycline and fluoroquinolone resistance in E. coli and Salmonella. Likewise, ALI (2020) noted the predominance of methicillin-

resistant Staphylococcus aureus (MRSA) among bovine mastitis cases in Punjab. At the international level, WHO and FAO documents have reiterated the dangers of AMR spreading in countries where antibiotics can be purchased without prescriptions and with restricted veterinary surveillance. This study further supports this evidence and contributes local evidence from the livestock production sector in Pakistan.

In theory, this study contributes to the One Health framework by demonstrating that human, animal, and environmental health systems are interconnected in the emergence, selection, and transmission of antimicrobial resistance (AMR) (Gonçalves, 2023). Based on practical considerations, they emphasize the pressing need for policy changes. These comprised enacting stringent antibiotic laws with a prescription-only policy, funding AMR awareness, and scaling up farmer training. In addition, the results highlight the importance of AMR surveillance in national livestock health programs. The data also indicate that promoting diagnostic testing over antibiotic administration may decrease the inappropriate use of broad-spectrum antibiotics in veterinary medicine, just as it would in human healthcare (Tang et al., 2017).

One of the advantages of this investigation is that it is based on two different methods: empirical microbiological data and survey results. This afforded a holistic perspective on the biological and behavioral factors that drive antimicrobial resistance (AMR). The regional representativeness of the sample (Punjab, Sindh, KP) enhances the applicability of the findings to the diverse agrarian regions of Pakistan. However, some limitations should be acknowledged. The study used self-reported survey data, which are subject to recall and social desirability biases (Bryman, 2001). Furthermore, limited resources prevented all isolates from being further characterized for resistance by genetic testing (e.g., PCR or whole-genome sequencing), thus limiting molecular information. No seasonal changes in resistance patterns were considered, as observations were accumulated over short periods.

Further research is needed to investigate seasonal variations in the emergence of antimicrobial resistance (AMR) over an extended period. Analysis of resistance genes at the molecular level should be included to gain a greater understanding of resistance mechanisms (Marshall & Levy, 2011). Longitudinal studies are also needed to ascertain the effectiveness of intervention programs (e.g., AMR training programs or prescription policies) over time. Moreover, investigating the interface of human-animal transmission flow, specifically within rural households that have regular contact with livestock, may provide a greater understanding of the transfer of zoonotic antimicrobial resistance (AMR). This study highlights the conundrum between the misuse of antibiotics, lack of awareness, and pathogen resistance in the livestock sector of Pakistan, underscoring the importance of harmonized One Health interventions to tackle the burgeoning challenge of antimicrobial resistance (AMR).

4. CONCLUSION

The main results of this study reveal that antimicrobial resistance (AMR) is widespread in the livestock sector of Pakistan, with Staphylococcus aureus in dairy cattle and Escherichia coli and Salmonella spp. in poultry exhibiting high resistance rates to frequently used antibiotics. These findings support the proposed hypothesis that the irrational use of antibiotics, coupled with low awareness of antimicrobial resistance (AMR), are significant factors contributing to resistance patterns, which can offer insight into veterinary and human health practices within the One Health concept. These results have significant implications for public health, veterinary policy, and antimicrobial resistance (AMR) stewardship, including the need for prescription-only regulations, farmer education, and integrated AMR surveillance. Although this study contributes to the accumulating evidence on zoonotic resistance in low-resource settings, it also highlights ongoing shortcomings in molecular profiling of resistant genes and long-term monitoring of antibiotic usage practices. Future studies should employ genomic-level investigations and longitudinal designs to further understand the seasonal trends and chain of transmission. However, this study was cross-sectional, relied on self-reported data, and was restricted to a specific area; these limitations suggest areas for further research. In summary, this study provides a critical evidence base for the dynamics of antimicrobial resistance (AMR) in Pakistan's

livestock sector, making explicit the need for multi-sectoral action and a basis for transformative interventions in both research and practice.

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