Toxics Link
for a toxics-free world

CELEBRATING 25 YEARS
1998-2023

WORLD ANIMAL PROTECTION

Poultry's Pill Problem: ANTIBIOTICS AND ITS ENVIRONMENTAL CONCERNS

In collaboration with
World Animal Protection

About Toxics Link:

Toxics Link is an Indian environmental research and advocacy organization set up in 1996, engaged in disseminating information to help strengthen the campaign against toxic pollution, provide cleaner alternatives and bring together groups and people affected by this problem. Toxics Link's Mission Statement - "Working together for environmental justice and freedom from toxics. We have taken upon ourselves to collect and share both information about the sources and the dangers of poisons in our environment and bodies, and information about clean and sustainable alternatives for India and the rest of the world." Toxics Link has unique expertise in areas of hazardous, medical and municipal wastes, international waste trade, and the emerging issues of pesticides, Persistent Organic Pollutants (POPs), hazardous heavy metal contamination etc. from the environment and public health point of view. We have successfully implemented various best practices and have brought in policy changes in the aforementioned areas apart from creating awareness among several stakeholder groups.

About World Animal Protection (WAP):

World Animal Protection is a globally operating organization committed to safeguarding animals from suffering caused by human activities. Operating in over 50 nations, our mission encompasses various facets of animal welfare, including farming, disasters, wildlife, and community welfare.

Within farming, we advocate for improved standards and the eradication of cruelty in food production, engaging with governments, corporations, and consumers to promote humane practices. In the wild, we focus on preserving endangered species, combating wildlife trafficking, and fostering responsible tourism practices that do not harm wildlife.

Through research, advocacy, and public awareness campaigns, World Animal Protection strives to foster a world where animals are treated with compassion & dignity, and their needs are integrated into all aspects of human life aiming towards a future where animals are valued and protected.

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Poultry's Pill Problem:
Antibiotics and its Environmental Concerns

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Executive Summary

Antimicrobial Resistance is one of the biggest threats to global health, endangering both human and animal health, the environment, food security, economic development and equity. According to the World Health Organization (WHO), around 1.27 million deaths occurred from drug-resistant infections in 2019. Today, antibiotics are globally used for therapeutic purposes, prophylactic measures, and as growth promoters in commercial animal production systems. The use of antibiotics in chickens is expected to triple in India by 2030 compared to 2015 as a consequence of the increasing consumer demands and intensive farming practices.

90%
of the total antimicrobials used in farms are released to the environment through animal excreta (urine and faeces)

The unregulated use of antibiotics in poultry may have serious implications for developing economies, especially for India, the biggest consumer of antimicrobials in the world. This includes a possible increase in export rejections and trade loss due to the stringent import policies in countries like the EU on the use of non-therapeutic antimicrobials for animal/animal-based products. There is ample literature linking the indiscriminate use of antibiotics with the rapid development of Antimicrobial Resistance (AMR) in various livestock sectors, including poultry and aquaculture in India.

The studies have found that about 90% of the total antimicrobials used in farms are released to the environment through animal excreta (urine and faeces). This release is a cause of concern as antimicrobials can persist for long periods, contributing to the development and selection of resistant bacteria, which are then open to infect both poultry and humans living nearby. There is an emerging concern globally on AMR, and India is working at various levels to minimise the risks associated with it. Toxics Link has developed a series of reports on antibiotic pollution and its impact on the overall environment.

In this context, to understand the existing scenario of antibiotic pollution from poultry on the overall environment and a factor in the development of AMR, Toxics Link, in collaboration with World Animal Protection, conducted a study on the presence of Antimicrobial Resistance Genes (ARG) in the environmental matrices surrounding poultry farms in Tamil Nadu and Andhra Pradesh. The lab-based study established that the environmental samples surrounding poultry factories contained facultative pathogens and opportunistic pathogens, along with ARGs against many of the antimicrobials classified under OIE and WHO's list of Critically Important Antimicrobials. These are classes of antimicrobials essential for the treatment of specific diseases, including those from non-human resources (e.g., Glycopeptides and Tetracycline). Furthermore, the study identifies gaps in the current regulatory framework for antimicrobial drugs and recommends amendments to the current system, highlighting the need for the adoption of newer policies and incentives against the indiscriminate use of antimicrobials in the poultry sector.

This study identifies gaps in the current regulatory framework for antimicrobial drugs and recommends amendments to the current system

Acknowledgements

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We would also like to express special thanks to **Dr Vijay Pal Singh** (CSIR-IGIB, New Delhi) for reviewing this report and providing us with his valuable feedback. The report findings were discussed in the webinar "Antimicrobial Resistance in Poultry" conducted on 21st July 2023. The comments and feedback received from the experts, particularly, **Dr Robin Paul** (Vet, Govt. of Kerala), **Dr Sudhi Rangorath** (Vet, Govt. of Kerala), **Dr. Anand Kumar** (Professor, NTR College of Vet Science, Andhra Pradesh), **Mr Satya Sivaraman** (ReAct Asia Pacific) were instrumental in improving this report.

1

Introduction

Antibiotics are natural, semisynthetic, or synthetic substances that interfere with the growth or survival of bacteria and are used to prevent or treat associated infections. The development of antibiotics is touted as one of the greatest accomplishments of modern medicine due to their ability to tackle bacterial infections. It has contributed to an increase in the average life expectancy of humans and animals, the control of infectious diseases, and a reduction in morbidity and mortality.[1]

However, the extensive use of antibiotics has led to antibiotic resistance by contributing to the emergence and dissemination of multidrug-resistant microorganisms. It is estimated that with the current rate of development of antimicrobial resistance (AMR) and microbes, the mortality rate caused by resistant-related infections is expected to surpass the mortality rate due to cancer by 2050.[2] In the year 2000, the World Health Organization (WHO) classified AMR as a global public health concern. In 2015, the World Health Assembly (WHA), the decision-making body of the WHO, adopted a global action plan on AMR. This action plan highlighted the need for an effective One Health approach to tackle this issue and coordination among several sectors, including human and veterinary doctors, farmers, economists, environmentalists and informed consumers.[3]

Amid the challenge of global food security driven by population growth, the need for sustainable food production systems becomes significant. There is an increasing demand for meat, with meat consumption increasing more than 4-fold in the last 50 years.[4] According to the FAO, the production of poultry meat accounted for almost 40% of global meat production in 2020.[5] As a result, there has been a global trend towards adopting intensive farming methods, which result in increased transmission of infections, including zoonotic diseases, impacting both animal well-being and productivity.[6] Consequently, compromised animal welfare is often attributed to overcrowded and stressful living conditions, limited access to natural behaviours, and the extensive use of antibiotics to mitigate disease outbreaks within these intensive farming systems.

In addition to the concerns related to animal welfare and food safety, the increase in meat production has led to concerns regarding production sustainability and public health. Intensive animal farming can lead to an increase in greenhouse gases, contamination of environmental matrices (such as drinking water), the dissemination of antimicrobial drug resistance, and the emergence and re-emergence of zoonotic diseases.[7]

Thus, the intensive animal farming activities and the use of antimicrobials (such as antibiotics) to safeguard the health of animals and animal products have contributed to the development and spread of AMR[8]. In this context, this study aims to focus on the contribution of the poultry sector to the mounting stress of AMR in India.

Food animals and antimicrobial resistance

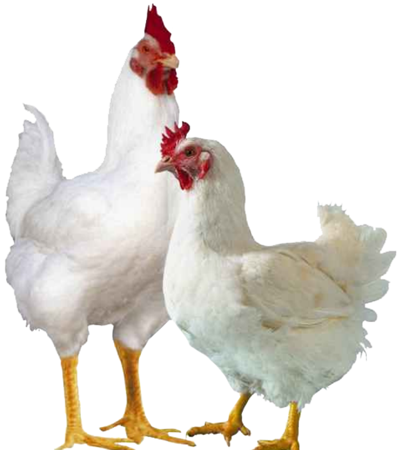
2

2.1 Use of antibiotics in food animals, including poultry

Antibiotic use plays a key role in selecting resistant bacteria, with both community and hospital environments serving as the primary breeding grounds for their emergence in human health[9]. However, the use of antibiotics in animals has further contributed to the global issue of AMR. Antibiotics are not solely used for therapeutic purposes in food animal production and aquaculture but also for disease prevention (metaphylactics and prophylactics) and growth promotion. Metaphylactic use involves the treatment of the entire group when a single animal shows disease symptoms, while prophylactic use involves the administration of sub-therapeutic doses to prevent the impact of stress-induced responses that typically pave the way for infectious diseases.[10] The Antibiotic Growth Promoters (AGPs) are antibiotics administrated at sub-therapeutic doses to modify the intestinal microbiota of animals to achieve faster growth and weight gain. This extensive use of antimicrobials in food-producing animals and fish is one of the reasons for the spread of AMR.[11]

The efficacy and cost-effectiveness of the majority of antibiotics have led to their indiscriminate usage. As a result, the misuse and overuse of these compounds promoted the establishment of microbial reservoirs carrying AMR determinants in livestock, including poultry. As some of the antimicrobials applied to animals are the same as those administered to humans, the spread of AMR poses a serious threat to the effective treatment of serious infections in humans, leading to higher medical costs, prolonged hospital stays and increased mortality.[12]

The application of AGPs started in 1951, when the US Food and Drug Administration (FDA) approved the use of antibiotics as animal additives without prescription, followed by the EU countries.[13]



Though the exact mechanism for the enhanced performance observed by the use of AGPs is not well understood, four hypotheses have been proposed[14]: (a) Prevention of nutrient depletion due to competitive bacteria; (b) Intestinal thinning due to antimicrobials could be increasing nutrient absorption; (c) Decrease in toxins released by intestinal microorganisms; (d) reduction in the incidence of subclinical intestinal infections. However, the use of AGPs has contributed to the rise and spread of AMR in the intestinal microbiota[15], prompting some countries to ban their use in animals.

2.2 Transmission of AMR from food animals

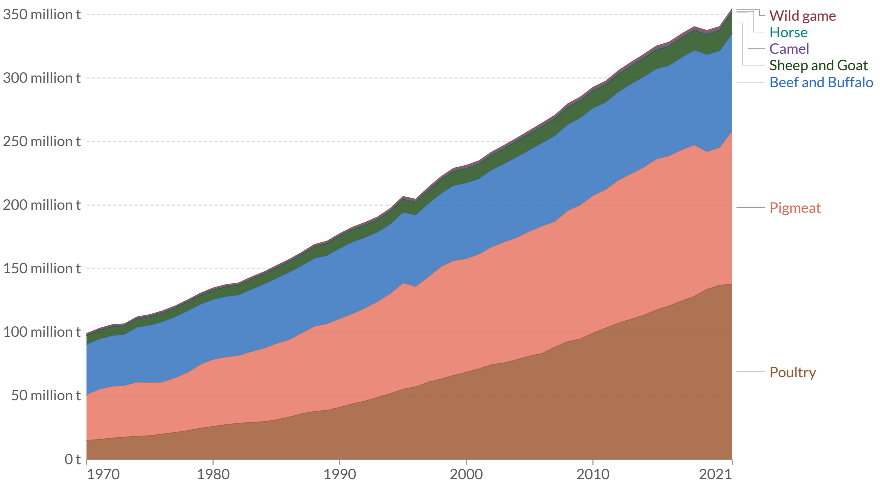
AMR has the potential to spread across the food chain through various pathways, including both direct and indirect interactions among different actors and environments[16]. These pathways are also recognized as the routes for the transmission of zoonotic diseases. Direct contact can occur when humans come into contact with resistant bacteria present in animals or their biological products, such as urine, faeces, blood, saliva and semen. For example, veterinarians, farmers, farm workers, food handlers, etc. have direct contact with the food animals and can have a higher risk of being infected with resistant strains.[17] Alternatively, indirect contact includes the handling and consumption of contaminated food products, such as meat and eggs.[18]

In addition, a large fraction of antibiotics is not degraded, or transformed into inactive compounds by animals or humans, and their activity is retained even after being excreted in urine and faeces. These antibiotic residues can accumulate in soils, wastewater and manure, leading to significant impacts.[19] Hence, the dissemination of resistant bacteria and antibiotic residues via food and animal waste turns the environment into a significant reservoir of AMR.[20] Intensive industrial farming produces large amounts of manure and other waste, and this mismanaged waste can be a significant source of contamination, including AMR genes, as it is well-known that the manure from animal farms, including poultry litter, is used as a fertiliser for agriculture and gardening. Therefore, soil in which these products have been used as a natural form of fertiliser can also become a reservoir of resistant organisms.[21] In addition to commensal and environmental bacteria, foodborne pathogens also carry AMR genes.[22]

3

Global Scenario and Regulations on antibiotic usage in the poultry sector

Poultry meat production has rapidly increased over the last 50 years. It accounts for approximately 33% of the overall meat production globally (see Figure 1). [23] Between the years 2020 and 2030, meat production is expected to be driven by the poultry sector and is projected to account for 50% of all additional meat produced within this period.



Data source: Food and Agriculture Organization of the United Nations OurWorldInData.org/meat-production | CC BY
Note: Total meat production includes both commercial and farm slaughter. Data are given in terms of dressed carcass weight, excluding offal and slaughter fats.

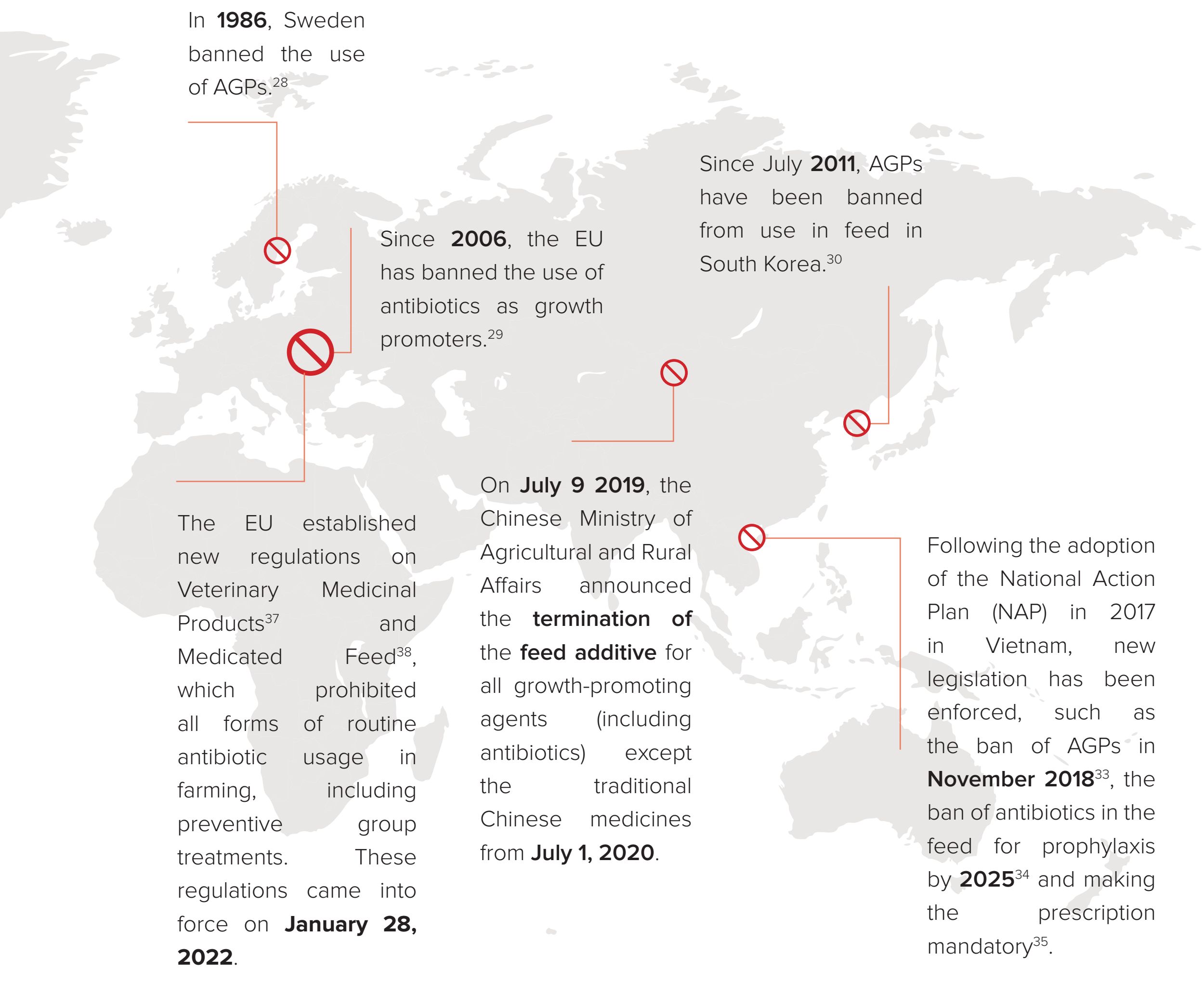
Figure 1: Overall meat production by livestock type in the World from 1971 to 2021[24]

The USA, Brazil, the European Union and China are the leading producers of poultry meat globally.[25] 14.1 million tonnes (37.5%) of the global meat trade volume (37.6 million tonnes) represent poultry meat exported by these leading producing countries, making chicken the most exported meat. This highlights the substantial economic significance of the poultry industry as a global commodity. There is a significant surge (725%) in the projected demand for poultry meat in South Asia by 2030, especially in countries such as India, where poultry meat consumption is anticipated to increase from 1.05 to 9.92 million tonnes per year over the next three decades.[26]

Owing to the rise in demand for livestock products globally, the number of antimicrobial drugs used in livestock is also expected to increase by 67% by 2030. The number of antimicrobials used in livestock is estimated to nearly double in countries such as the BRICS countries (Brazil, Russia, India, China and South Africa).[27] Therefore, it is important to regulate antibiotic usage in poultry to combat the development of antibiotic-resistant bacteria. Numerous nations have proactively initiated a range of policies, bans and regulations to tackle this challenge effectively.

In **2015**, the US introduced the veterinary feed directive, whereby the use of drugs on the veterinary feed directive list is permitted only under the professional supervision of a licensed veterinarian.31 Further, the U.S. Food and Drug Administration (FDA) banned the use of antibiotics as feed supplements with effect from **January 1, 2017.**[32]

In **January 2020**, a few antibiotics (lincomycin, tiamulin and tylosin) were prohibited as growth promoters in animals destined for human consumption in Brazil.[36]



In **1986**, Sweden banned the use of AGPs.[28]

Since **2006**, the EU has banned the use of antibiotics as growth promoters.[29]

Since July **2011**, AGPs have been banned from use in feed in South Korea.[30]

The EU established new regulations on Veterinary Medicinal Products[37] and Medicated Feed[38], which prohibited all forms of routine antibiotic usage in farming, including preventive group treatments. These regulations came into force on **January 28, 2022**.

On **July 9 2019**, the Chinese Ministry of Agricultural and Rural Affairs announced the **termination of** the **feed additive** for all growth-promoting agents (including antibiotics) except the traditional Chinese medicines from **July 1, 2020**.

Following the adoption of the National Action Plan (NAP) in 2017 in Vietnam, new legislation has been enforced, such as the ban of AGPs in **November 2018**[33], the ban of antibiotics in the feed for prophylaxis by **2025**[34] and making the prescription mandatory[35].

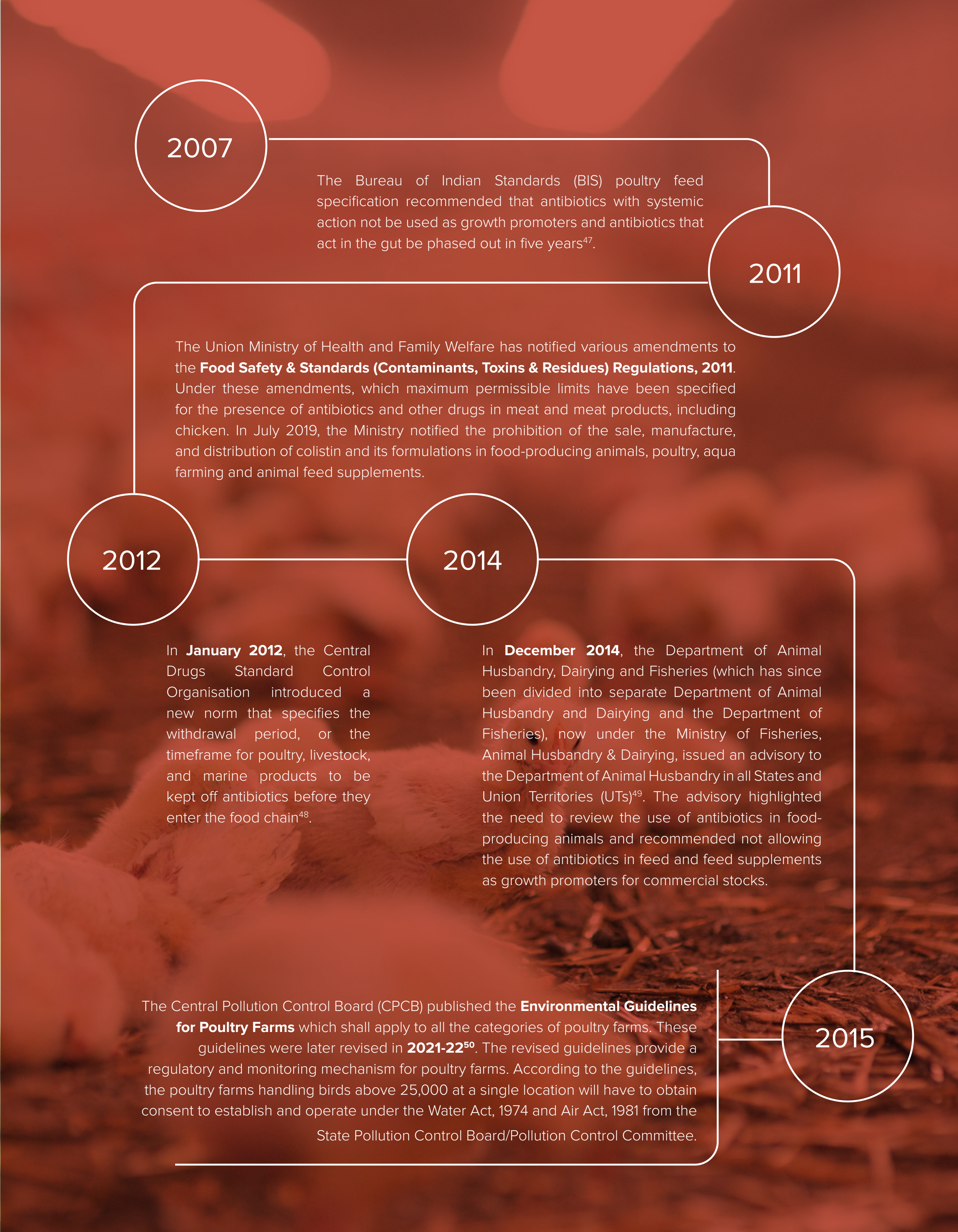
4

Poultry industry landscape and Policy framework in India

India is reliant on its poultry production for meat and eggs. Despite having the largest population of livestock in the world[39], according to the 20th Livestock Consensus of India, the total poultry population (851.81 million) far outweighs the total livestock population (535.8 million)[40]. Making the country the world's third-largest producer of eggs and the fourth-largest in terms of chicken meat production by volume[41]. Poultry meat production also constitutes about 50% of the country's total meat production. Broiler production (poultry used for meat) is mainly concentrated in the states of Tamil Nadu, Andhra Pradesh, Maharashtra, Uttar Pradesh and Telangana.[42]

Alongside the positive growth in the sector, there are growing concerns over the indiscriminate use of antimicrobials in poultry that has become rampant in India. The use of antimicrobials in the country is often seen as an effective and cheap method for animal care. It is applied to reduce disease risk, as a growth promoter and for sub-therapeutic treatment (in water). [43] On average, the country administers antimicrobials at a rate much higher than the world's average - 3% of the total global consumption of antimicrobials in food animals[27]. The intensity of antimicrobial usage (AMU), the number of milligrams administered per kilogram of meat in India, is expected to reach up to 40% more than the global average by the year 2030.[44] In 2021 alone, India's antimicrobial consumption for animal use reached a peak of 2,160 tonnes, with a new peak predicted to be reached in 2030[45]. The country has become a site of one of the world's highest rates of antimicrobial resistance, both in humans and food animals.[46]

Though there are no regulations on antibiotic usage in the poultry sector, there are advisories and guidelines.



2007

The Bureau of Indian Standards (BIS) poultry feed specification recommended that antibiotics with systemic action not be used as growth promoters and antibiotics that act in the gut be phased out in five years[47].

2011

The Union Ministry of Health and Family Welfare has notified various amendments to the **Food Safety & Standards (Contaminants, Toxins & Residues) Regulations, 2011**. Under these amendments, which maximum permissible limits have been specified for the presence of antibiotics and other drugs in meat and meat products, including chicken. In July 2019, the Ministry notified the prohibition of the sale, manufacture, and distribution of colistin and its formulations in food-producing animals, poultry, aqua farming and animal feed supplements.

2012

In **January 2012**, the Central Drugs Standard Control Organisation introduced a new norm that specifies the withdrawal period, or the timeframe for poultry, livestock, and marine products to be kept off antibiotics before they enter the food chain[48].

2014

In **December 2014**, the Department of Animal Husbandry, Dairying and Fisheries (which has since been divided into separate Department of Animal Husbandry and Dairying and the Department of Fisheries), now under the Ministry of Fisheries, Animal Husbandry & Dairying, issued an advisory to the Department of Animal Husbandry in all States and Union Territories (UTs)[49]. The advisory highlighted the need to review the use of antibiotics in food-producing animals and recommended not allowing the use of antibiotics in feed and feed supplements as growth promoters for commercial stocks.

2015

The Central Pollution Control Board (CPCB) published the **Environmental Guidelines for Poultry Farms** which shall apply to all the categories of poultry farms. These guidelines were later revised in **2021-22**[50]. The revised guidelines provide a regulatory and monitoring mechanism for poultry farms. According to the guidelines, the poultry farms handling birds above 25,000 at a single location will have to obtain consent to establish and operate under the Water Act, 1974 and Air Act, 1981 from the State Pollution Control Board/Pollution Control Committee.

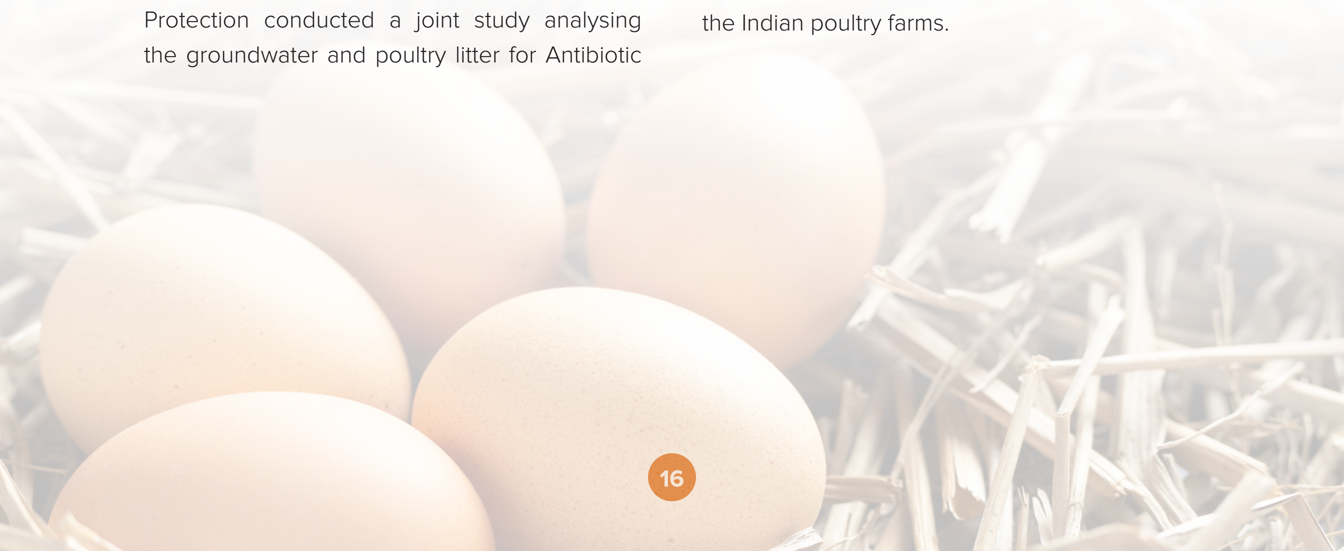
5

Research Study

5.1 Rationale of the study

It has been estimated that up to 90% of the antimicrobials used at the farm level are released into the environment through animal **excreta** (urine and faeces).[51] This release is cause for concern, as the antimicrobials released can persist for long periods, contributing to the development and selection of resistant bacteria. When the microorganisms and their resistant genes enter the environment, they can persist and eventually stabilise in the **microbial community**.[52] Thus, the **poultry litter** (mixture of bedding material, manure, feathers and spilled feed) produced on the farms has been proven to be a prime reservoir of antibiotic resistance and related genes.[53]

In this context, Toxics Link and World Animal Protection conducted a joint study analysing the groundwater and poultry litter for Antibiotic Resistance Genes (ARGs). ARGs are genetic elements that not only provide resistance against antimicrobials present, but are also mobile elements that can be passed on from one microorganism to another through the means of transduction, conjugation and transformation. This allows the spread of resistance throughout the bacterial population in a given area (horizontal transmission)[54]. Groundwater is a critical resource and is susceptible to poultry-derived contamination with antibiotic-resistant bacteria and genes. These can have far-reaching consequences, affecting both animal and human health. Thus, by studying the ARGs in both groundwater and poultry litter, we can elucidate the influence of antibiotic usage in poultry on the overall pool of ARGs within the environment of the Indian poultry farms.



5.2 Objectives of the study

The major goal of this study was to investigate the occurrence and implications of ARGs in poultry litter and the groundwater near poultry farms.

The objectives of this study include:

1. Understanding the presence of ARGs in poultry litter and highlighting the role of poultry litter as a reservoir for antibiotic resistance and related genes
2. Assessing the presence of ARGs originating from poultry litter in groundwater near poultry
3. Building upon the findings, formulate recommendations for the poultry sector and government to limit the misuse of antibiotics and minimise the emergence and spread of ARGs

This study aims to comprehensively investigate the multifaceted impact of AMU on animal production within the context of AMR. Specifically, the objective is to assess the interconnections between human, animal and environmental health aspects. By focusing on the enhancement of animal welfare, improvements in farm biosecurity, and the significant reduction of non-therapeutic treatments on animals, the study seeks to elucidate the holistic implications of the One Health approach. Furthermore, the study aims to fill the existing knowledge gap by exploring the links between AMU in factory farms and the social and health burden of AMR. Ultimately, the objective is to contribute valuable insights that can inform evidence-based policies and interventions addressing AMR at the intersection of human, animal, and environmental health.

The study findings will be crucial in informing strategies, policies, and best practices to address and manage the challenges posed by ARGs originating from poultry farming. We believe that this study will add to the growing scientific evidence and reinforce the need for urgent action on AMR.

5.3 Methodology

For the isolation and analysis of possible ARGs in the poultry environment, the method of metagenomic analysis was utilised. The whole 'metagenome' sequencing of DNA offers a comprehensive view of the genomes of the total microbial community present in the natural environment. This approach facilitates the study of both culturable and non-culturable bacteria by bypassing the need for isolation and laboratory cultivation of microorganisms. Whole genome sequencing overcomes the limitations of traditional culture-dependent approaches and phenotypic tests, i.e., disk diffusion for AMR.[55] DNA directly isolated from environmental samples can broaden the understanding of the structure, gene/species richness and distribution, as well as the functional and metabolic potential of a microbial community.[56]

Applying this approach to explore AMR in different microbial communities can help in identifying known and novel resistance genes and mobile genetic elements, i.e., plasmids, integrons, transposons, and pages.[57] This information can be useful in formulating new policies for infection and prevention control measures, thereby reducing the incidence of infection and optimising the use of antibiotics in different sectors. In addition, it can provide improved awareness and understanding of antibiotic resistance as a whole.

In this study, the metagenomes were extracted from the litter and water samples and then sequenced. The genes identified in the 11 samples were screened against the Comprehensive Antibiotic Resistance Database (CARD) to obtain a list of ARGs in each sample with their corresponding gene name, ARO name, drug class, AMR gene family and resistance mechanism (see Figure 2).

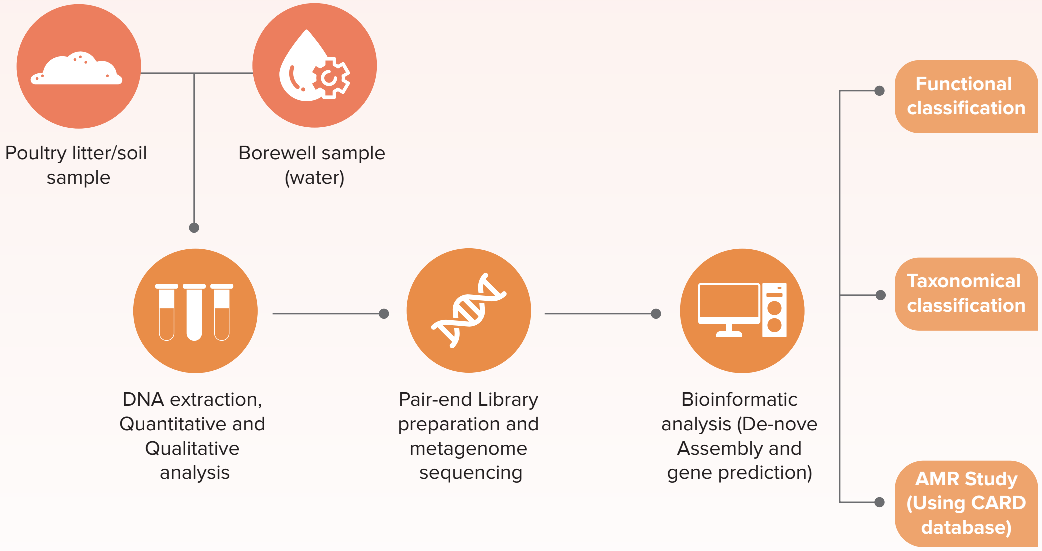


Figure 2: Metagenomic analysis of the collected sample

5.4 Sampling

In this study, 14 samples in total, including poultry litter samples and borewell water (groundwater samples), were collected from six poultry farms in two Indian states (Tamil Nadu and Andhra Pradesh) and transported to the Genomics Laboratory in Bengaluru, Karnataka, for metagenomics analysis (whole metagenome sequencing). The poultry farms were selected based on their proximity to the city and the size of the bird population (at least 10,000 birds including all ages). Details of the poultry farm, including location, type, and size, and details of the samples collected at each are presented in **Table 1**. Before sampling, a qualitative survey was conducted to gather in-depth information and insights about the poultry farm from the owners, especially regarding the use of antimicrobial drugs.

Table 1: Details of the samples collected from Andhra Pradesh (AP) and Tamil Nadu (TN)

Location	Type & Size of farm	Sample ID collected	Additional observations
Gosala village, Krishna district, AP	Broiler, 15,000 birds (27 days old – 5,000 flocks 36 days old – 10,000 flocks)	VD-1 (poultry litter- 27 d) VD-2 (poultry litter-36 d) VD-5 (borewell water)	Antimicrobials Furazolidone, Neomycin (aminoglycoside), Doxycycline (tetracycline) were found on site.
Peda Ogirala village, Krishna district, AP	Broiler, 20,000 birds (All birds were 32 days old)	VD-6 (borewell water), VD-3 (32 days poultry litter) VD-4 (32 poultry litter sample from different shed)	Farm integrated with a major food company
Medikonduru Village, Guntur district, AP	Broiler, 10,000 birds (Flocks were 30 days old)	VD-7 (borewell water)	Self-managed, independent poultry farm

Pilliappampalayam, Coimbatore (Annur rural block), TN	Combined Layer & Broiler Layer – 60,000 birds Broiler – 17,000 (13 days)	C-1 (borewell water); C-4 (poultry litter-layer farm)	-Layer farm was independent and the broiler was integrated with a food company. -Broiler farm also housed a few birds of indigenous variety on its premises. -A litter sample was collected from the layer farm section as the broiler farm was leased to others and they refused to provide the sample
Kembanaickenpalayam Village, Coimbatore (Annur rural block), TN	Broiler farm 1 - 6,000 birds Broiler farm 2 – 10,000 birds (13 days old)	C-2 (borewell water) C-5 (soil besides broiler farm 1) C-6 (poultry litter-fully grown)	-Broiler Farm 1 was integrated with one food company and Broiler Farm 2 was integrated with another food company. Both the farms were located next to each other with hardly 50 m distance -Shed in the Broiler Farm 1 was vacant as the birds were sold just 4 to 5 days earlier. As litter was not available, soil beside the shed was collected. -Broiler farm 2 had 13 days old birds. However, a small amount of poultry manure from fully grown birds was stored, which was sampled for the study -Borewell water from the two farms was collected and mixed as both were located close to each other.
Kuppayapalayam village, Coimbatore (Annur rural block), TN	Broiler, 10,000 flocks (13 days old)	C-3 (borewell water) C-7 (13 d poultry litter)	-The farm was associated with Shanti feeds and Sugana foods in the past and is now integrated with MBS feeds.

The genes predicted by the whole metagenome sequencing were considered for taxonomic classification and screened against the Comprehensive Antibiotic Resistance Database (CARD) for the identification of ARGs.

5.5 Results and Discussions

5.5.1 Taxonomic abundance

After the metagenomic DNA extraction from the litter and water samples, more than 2.5 million genes were annotated, out of which 45,339 were CARD-annotated genes. This was from 11 samples collected from Vijayawada (VD1-7) and Coimbatore (C4-7). The three water samples from Coimbatore (C1, C2, C3) contained no DNA. This can be attributed to the steep depth of the well from which the samples were collected. The water samples collected were completely sterile.

Of the 11 samples that did contain genetic materials, all the samples had the highest concentrations of bacteria (as the super kingdom), ranging from 77% to 92% in Vijayawada to 79% to 82% in Coimbatore samples. At the phylum level, Actinobacteria (19–44%), Firmicutes (7–44%), Proteobacteria (11–27%) and Bacteroidetes (5–13%), were the dominant phyla in all the litter samples of both regions and the soil beside the shed mixed with litter in Coimbatore (C5), with the four phyla contributing to 67–79% of the total bacteria. Proteobacteria (57–89%) was the dominant phylum in water samples from Vijayawada (VD5–7). This indicates that major ARG transfer in litter samples is undertaken by Actinobacter, Firmicutes, Proteobacteria and Bacteroidetes. Similar trend was observed in a study conducted by Xu et al. and Liu et al. in 2023 which analysed ARGs in poultry manure.[58]

Litter samples were a homogenous mixture of different genera. Few of the genera with slightly higher abundance were Microbacterium (2% in C4), Brachybacterium (1–7.5% in C5–7 samples, 7–10% in VD1–4 samples), Brevibacterium (7–9% in VD1–4 samples) and Corynebacterium (4.5–8% in VD1–4 samples). Pseudomonas was the predominant genus in one of the water samples (20% in VD7), while it was not significantly predominant in the other two water samples.

Dermabacteraceae (4.61% - 6.13%), Staphylococcaceae (4.34% - 14.57%) and Corynebacteriaceae (2.29% - 5.02%) were the most abundant families found in the C5–7 litter samples. Flavobacteriaceae, Bacillaceae, Microbacteriaceae, and Alcaligenaceae were dominant families in the C4 litter sample. Dermabacteraceae, Staphylococcaceae, Brevibacteriaceae, Corynebacteriaceae, Lactobacillaceae, and Dietziaceae were the predominant families in the VD1–4 litter samples. Pseudomonadaceae and Comamonadaceae families were present in all three water samples (VD5–7), with a high abundance of 20% and 18% in VD7, respectively. Moreover, Sphingomonadaceae and Rhodobacteraceae were abundant in VD5 and VD6 samples.

Abundance at the species level

Detected species can be classified into pathogenic and non-pathogenic organisms. We specifically focused on Staphylococcaceae, Enterococcaceae and Enterobacteriaceae, because these families contain obligate and facultative pathogens. Several pathogenic species were identified within the three targeted families described above. Escherichia coli was the most dominant Enterobacteriaceae species in the C5–7 samples, while Klebsiella pneumoniae was the most dominant in C4. All the litter samples from Vijayawada (VD1–4) had a high abundance of Escherichia coli. One of the farms (VD1 and VD2) had also reported high mortality due to E. coli infections. A lower abundance of Klebsiella pneumoniae was also observed in all these samples. These samples had very low levels of Enterococcus species.

Most Enterococcus species were non-pathogenic species; however, significant levels of the zoonotic pathogen Enterococcus hirae, which is involved in growth depression and endocarditis in chickens, and blood infections in humans were detected in C5 and C7. Moreover, a lower abundance of the opportunistic enteric human pathogen Enterococcus faecium was also detected in these samples. Non-pathogenic species, including Jeotgalicoccus spp. and Staphylococcus sp. dominated Staphylococcaceae in the C5–7 and VD1–4 samples. However, a significant level of the opportunistic pathogen Staphylococcus aureus was also detected in C5, and a low level was detected in C6 and V4. Samples VD3 and VD4 showed a significant presence of C. perfringens, which is the causative agent of necrotic enteritis and gangrenous dermatitis in broiler chickens.

The litter from livestock production has been associated with the contamination of water resources via runoff from land application, leaching into groundwater and manure spills.[59] Although the groundwater samples were dominated by other species, they demonstrated a significant presence of Escherichia coli and Staphylococcus aureus.

5.5.2 Analysis of Antibiotic Resistance Genes detected

The genes identified in the 11 samples were screened against the Comprehensive Antibiotic Resistance Database (CARD). The CARD annotated gene counts ranged from 1591–7913, with a mean of 4121, conferring resistance to major classes of antibiotics including tetracyclines, peptides, glycopeptides, aminoglycosides, macrolides, fluoroquinolones, multidrug, etc. The litter samples in Coimbatore had a large variation in the gene counts, indicating a greater diversity and abundance of ARGs across samples. On the other hand, the gene counts in Vijayawada litter samples varied only within a small range (i.e., 3545–3845). However, a large variation was observed in three borewell water samples (1591–3710), as the presence of ARGs in groundwater can depend on various factors, such as the management practices on the farm, hydrogeological conditions, local environmental factors, etc. Thus, poultry can be an important reservoir of antibiotic resistance.

The relative abundance of multidrug ARGs was the highest across all samples comprising at 25-40% of ARGs. This was followed glycopeptides (18.3 ± 3.1%), tetracyclines (16.9 ± 5%), peptides (12.5 ± 1.7), aminoglycosides (11.4 ± 1.4%), and macrolides (9.1 ± 2.4%) were the most abundant (**Figure 2**). Other significant ARGs like rifamycin, phenols and sulphonamides were also observed, but they were present at a much lower concentration (1.4–3.7%).

The higher concentrations of tetracyclines, peptides and glycopeptides may be attributed to their historical use in food-producing animals. In addition to their therapeutic use, they were also previously extensively sold as feed additives for growth promotion (for example, Avoparcin, Bacitracin).[60, 61] Despite the introduction of new antimicrobials and changes in consumption patterns, these three main classes remain dominant across different farms and environmental matrices, which was even observed in the present study.

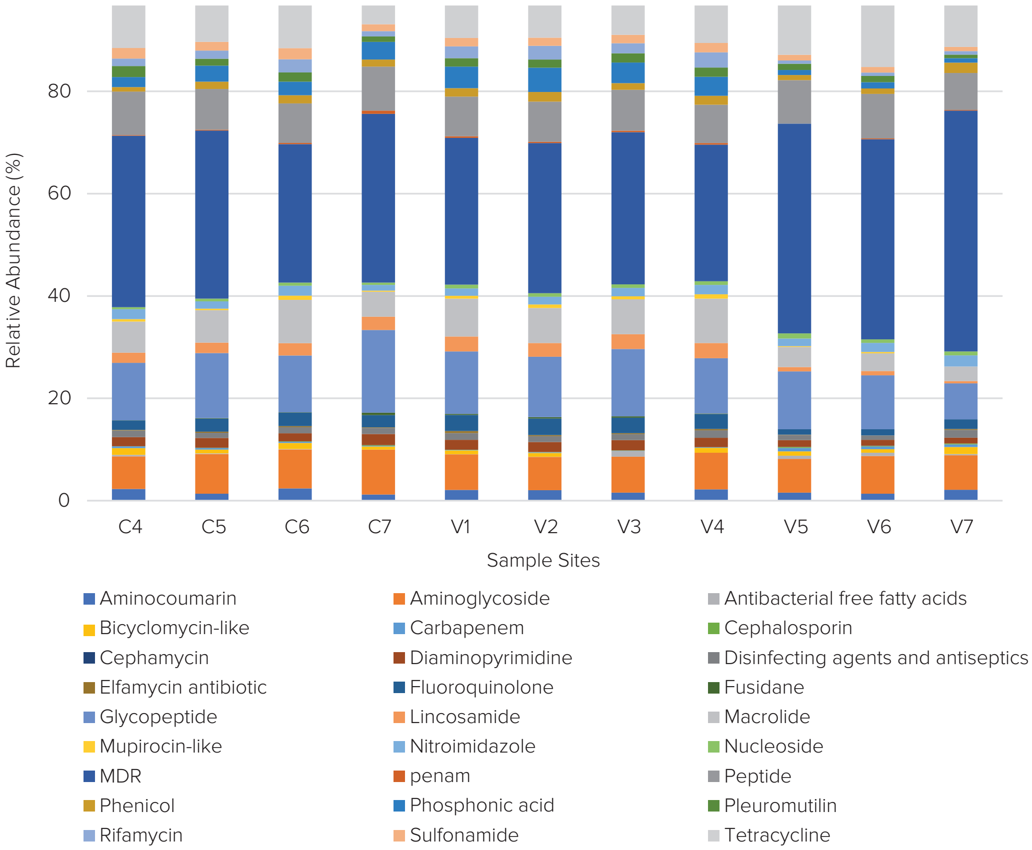


Figure 3: Relative abundance of antimicrobial resistant genes (ARGs) based on the antimicrobial classes. ARG types were mapped and corresponded to their respective antimicrobials.

Similarly, ARGs for drugs like macrolides, cephalosporins, fluoroquinolones, etc., which are very important/critical for both human and animal consumption, were present in higher abundance than the other groups of antimicrobials, in this study, whereas, ARGs predominantly used for veterinarian purposes (poultry) like Aminocoumarin, Elfamycin and Pleuromutilin were relatively lower. Aminocoumarin is commonly used in broiler farms against gram-positive cocci bacteria[62], Elfamycin as a growth promoter[63], and Pleuromutilin to prevent and control respiratory diseases[64]. This could indicate that these antimicrobials are either new to these poultry farms or are generally consumed less than relatively older ones.

Most antimicrobials for which the resistant genes were observed in the study can be classified under the new WHO's list of Medically important antimicrobials for human and veterinary medicine (**see Table 2**).[65, 66]

Table 2: Antimicrobials (for which resistance was detected) classified under WHO's new list of Medically Important Antimicrobial (MIA) drugs

Medically important antimicrobials					Not medically important
Authorized for use in humans only	**Authorized for both humans and animals**				Not authorized in humans
Class	**Categorizations of antimicrobials**				
	HPCIA	**CIA**	**HIA**	**IA**	
Mupirocin-like	Cephalosporins	Macrolides	Tetracyclines	Pleuromutilin	Aminocoumarins
Carbapenems	Phosphonic acid	Aminoglycosides	Nitroimidazoles		Bicyclomycins-like
Glycopeptides		Rifamycin (Ansamycins)	Sulfonamide		
			Lincosamide		
			Cephamycin		
			Fusidanes		

The study found a number of ARGs to critically important antibiotic classes. This is a matter of concern because when a drug is considered critically important, it suggests that the antibiotic class could be one of the few available options for treating infections in humans. These infections might originate from nonhuman sources or involve bacteria that have acquired resistance genes. In India, many of these antibiotics are crucial for managing various infectious diseases and conditions, and some are reserved as last-resort treatments in hospital settings.

As shown in Figure 4, tetA (58), arlR, RanA, bcrA, oleC, and erfB were the most abundant genes observed in all the litter samples. The tetA (58) gene belongs to a major facilitator superfamily, which is one of the largest known transporter families and has been reported to confer resistance to tetracycline.[67] arlR gene has been identified in different strains of S. aureus and is known to regulate processes like adhesion, autolysis, multidrug resistance, and virulence.[68] RanA mainly confers resistance to aminoglycoside antibiotics.[69] bcrA has been frequently associated with bacitracin resistance in Bacillus licheniformis. Additionally, TxR (conferring tetracycline resistance) was the most abundant gene in the C4–7 samples and AbaF (resistance to Fosfomycin) and TaeA (conferring resistance to pleuromutilin antibiotic) were present in the VD1–4 samples.

Several genes conferring resistance to clinically important antibiotics, e.g., β-lactams (i.e., ampC, blaZ, mecA), were detected. vanC, which is frequently associated with multidrug resistance in opportunistic enterococci pathogens was also detected, indicating many of these strains may be resistant to vancomycin. Except for C4, aadA, an aminoglycoside resistance gene that is often associated with various pathogens, including E. faecalis and E. coli, was present in all the samples.[70, 71]

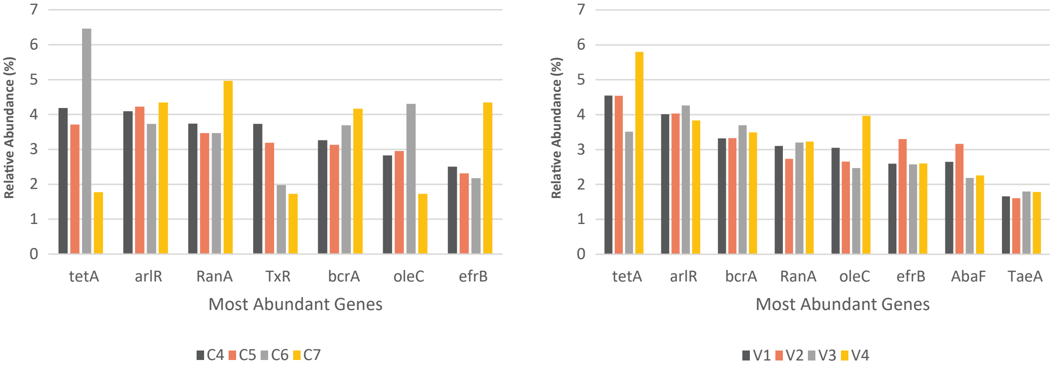


Figure 4: Most Abundant genes in the litter sample

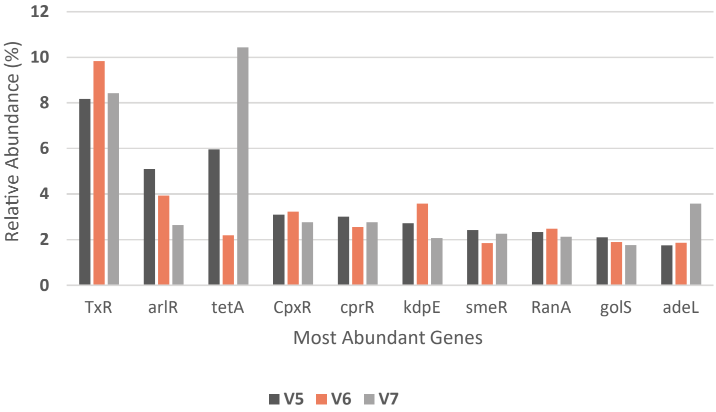


Figure 5: Most abundant genes in the water samples

The TxR gene was the most abundant in the Vijayawada water samples. Similar to the litter samples, arlR, tetA(58) and RanA were the most abundant genes in the groundwater samples. Less abundant genes such as CpxR, smeR, adeL and golS (**see Figure 5**) conferred resistance against multiple drugs, whereas cprR and kdpE conferred resistance against peptide and aminoglycoside drugs, respectively.

A similar study was conducted in Spain for the determination of ARGs in irrigation water of agricultural area with intensive use of poultry manure. Along with beta lactam resistant genes, the presence of genes such sul1-3 in water was detected which is consistent in our finding in the water samples in Vijayawada (VD-5,6,7)[72]. Study conducted by Zong et.al (2022) on the difference between ARGs in Broiler farms and layer farms detected high concentrations of aph (3') – Ia, aadA, fosB, qnrD, tetA in broiler farms.[73] When compared to our samples (broiler), genes such as fosD, tetA, aadA, aph (3') – Ia and aph (6')-Ia were detected, with tetA having the highest relative abundance.

Similar observation was made by Liu et al. (2024) in duck farm environment in south-east coastal China where researchers presented these ARGs as a point of distinction between soil samples and manure samples.

All the genes mentioned in this study have been observed, to some extent, in more than 300 important pathogens. For example, the oleC gene conferring macrolide resistance was observed in plasmids (mobile elements) of Acinetobacter baumannii, chromosomes of Bifidobacterium, and Pseudomonas species, etc74. This indicates that there is a constant movement of genetic material amongst different microorganisms. It could be from pathogen to pathogen, pathogen to non-pathogen, or just non-pathogen to non-pathogen, depending on the situation and exposure to the environmental matrices. Similarly, ARGs found in poultry microbiomes could be easily transferred into the surrounding environment, particularly in soil microbiomes, once the poultry litter is applied as manure in agriculture fields as the detected genes originated from mobile elements (e.g. sul4 genes). Such a scenario would be disastrous if these ARGs or antibiotic-resistant strains were to make their way into humans.

It should be noted that not all ARGs lead to antibiotic resistance. The presence of an ARG in an organism does not always mean that the organism will exhibit resistance to antibiotics. Several factors that influence the expression and functionality of ARGs, including the genetic context, regulatory mechanisms and the environment.[75]

It is also important to note that while ARGs themselves may not always result in resistance, the presence of these genes can provide a genetic reservoir that can contribute to the development of resistance in pathogens over time. The continuous and indiscriminate use of antimicrobials creates selective pressure that favours the survival and proliferation of bacteria carrying ARGs, leading to the emergence of resistant strains.[76]

Therefore, the presence of ARGs is a significant concern, and monitoring their dissemination is crucial for discerning their potential risks associated with antimicrobial resistance.

6

Limitations and Future Direction

There are very few studies on ARG profiling in poultry environments and litter in India. This study aims to draw attention to the potential of poultry in increasing cases of antimicrobial resistance in India. Such studies are a step towards action required to minimise the overuse/misuse of antibiotics in poultry. Therefore, as with most studies, the current design of the study is subject to its limitations. A few of these are as follows:

- **Small sample size and fewer sampling locations** - All robust research studies employ a large sample size to reduce overall variance between samples and enhance statistical metrics. This primary study, however, dealt with smaller samples due to monetary constraints. Hence, all results had to be made purely on a comparative basis.

- **Lack of sampling from non-poultry areas** - The ARGs may not always imply antimicrobial resistance in birds. Genetic context and environment need to be investigated further, especially in contrast to conditions dissimilar to poultry regions. Furthermore, additional sampling can be conducted on workers and nearby settlements to understand the transmission dynamics of resistance from manure to the environment to humans.

- **Technique constraints** - Not all ARGs lead to antibiotic resistance. Therefore, the presence of an ARG in an organism needs to be compounded with favourable conditions to see the effects. Several factors influence the expression and functionality of ARGs, including the genetic context, regulatory mechanisms and the environment. Therefore, additional dependent variables need to be taken into consideration.

- **Lack of knowledge on antimicrobials used** - A cocktail of ARGs was detected for antimicrobials used exclusively in humans and animals and for both humans and animals, confirming the source of the antimicrobials. However, a more direct link between the consumption of antimicrobials and their release as waste needs establishment. This will further assist in curbing the overconsumption of antimicrobials in poultry farms and the variety of antimicrobials in circulation.

- **Lack of research studies in India** - Studies on present antimicrobial consumption and the presence of antimicrobial genes in the poultry environment based in India are rare. Therefore, mere research comparisons from different reports from different countries are insufficient. Further studies are needed to develop databases, surveillance and strategies for AMR mitigation based on this primary research.

7

Insights and Recommendations

Minimising antibiotic use in food animal production is the most effective way to address resistance spread from animal farms. Overall, these findings indicate that banning non-therapeutic antibiotics in factory farm animals is crucial to prevent an economic and health crisis. Examples from husbandry systems that have phased out antibiotics or undergone testing suggest that removing prophylactics is possible with only minor reductions in productivity and health, which can be addressed through improved welfare conditions.[77,78] Sweden banned non-therapeutic antibiotics in 1986, and since then, the health, welfare, and production of animals have not been adversely affected[79]. However, measures have been continually undertaken to optimise the rearing and production systems, employing available techniques related to the number of animals, age grouping and planned production.

The ban in Sweden also led to the development of new rearing systems, such as piglets weaned on deep straw litter beds, late weaning at 30 days, large group cages, and the birth-to-slaughter system, where production occurs in the same pen from birth to slaughter. [72] Nonetheless, adjusting production systems, old buildings, feed, and pens is costly. Findings related to cost implications five years after the ban on non-therapeutic antibiotics in poultry meat showed that only the structure of variable costs changed, with no additional costs incurred.[80]

Similar to the pig industry, the phase-out of non-therapeutic antibiotics in poultry necessitated a reduction in stocking density, improved breed selection for feed efficiency, clean litter provision, and enrichment. Therefore, from a policy perspective, evidence suggests that governments and industries discontinuing the use of non-therapeutic antibiotics should provide the right incentives for improving feed quality, animal welfare, hygienic and sanitation management, reducing animal numbers, and using breeds that adapt to local environments, have strong immune systems, and can enhance the utilisation of available feed.

In India, there are key regulators like the Central Drugs Standard Control Organisation (CDSCO), their state-level regulators and implementor agencies like the State Departments of Animal Husbandry and Pollution Control boards to ensure the judicious use of antibiotics and minimise the risk associated with it.

Therefore, seeing the current state of framework, we suggest the following recommendations:

- There is an urgent need for the prohibition of non-therapeutic use of antibiotics for growth promotion and mass disease prevention. Antibiotics should only be used to treat sick animals, based on the prescription of veterinarians. Furthermore, penalties need to be established for non-compliance with antibiotic use guidelines.

- Antibiotics should not be allowed in feed and feed supplements. The government needs to come up with mandatory standards for animal feed. Moreover, they need to regulate the business of animal feed and feed supplements. Further there is a need to develop a national database for reporting antibiotic use data by food producers.
- It is essential to prohibit the use of last-resort antibiotics to treat multidrug resistance in humans for animals' use. (like colistin).
- Allocate research funding to develop alternative farming systems that do not rely on antibiotics for use in animal agriculture. Support research on innovative farming practices that reduce the need for routine antibiotic administration.
- Farmed animal welfare regulations should be introduced and enforced in line with the **FARMS initiative**[81] at minimum in recognition that improved animal health and welfare will allow for responsible reduction in antibiotic use.
- Good farm management practices and biosecurity guidelines should be followed to control infection and stress among the birds. To achieve this, there is a need for capacity building among small-scale farmers.
- Training and educating veterinarians and farm owners on the judicious use of antibiotics and maintaining high animal welfare to prevent infection. Develop and support antibiotic stewardship programmes to educate farmers on responsible antibiotic use.
- Periodic monitoring of antibiotic use and antibiotic resistance is necessary to create an integrated surveillance system for monitoring antibiotic use and trends of antibiotic resistance in humans, animals and the food chain. Implement systems for tracking and reporting antibiotic sales and usage in the agriculture sector.
- Introduction of incentive-based systems, like the one adopted by Sweden, wherein farmers from breeding and production farms would be incentivised for improving feed quality, animal welfare, hygiene and sanitation management, and for using breeds that adapt to local environments, have strong immune systems and can enhance the utilisation of available feed.
- The food producers need to create a mechanism to adopt labelling practices on antibiotics-use in animal food. The government may need to develop a regulation that mandate the industryto adopt labelling which will be accurate and visible
- The government needs to launch nationwide campaigns to educate consumers about the implications of antibiotic use in animal agriculture and reduce consumption of animal products. Provide resources for educational programmes in schools and communities to raise awareness.
- Incentivise and support farmers transitioning to sustainable and regenerative agricultural practices that reduce reliance on antibiotics. Develop programmes that reward farms for employing effective antibiotic reduction strategies.

- There is also a specific need to manage poultry waste to minimise risks associated with antibiotics.
- The environmental regulations for poultry farms and feed industries need to be strengthened with a focus on AMR.
- Untreated litter should be prohibited from further reuse. Only treated litter/manure should be allowed to be reused, as treatment processes are known to reduce ARGs.
- Specifically, poultry litter should not be allowed to be used as feed in aquaculture to prevent resistance spread across food animal production settings.
- There needs to be collaborative work with international organisations to share best practices and coordinate efforts to combat antibiotic use in agriculture on a global scale.
- SPCB needs to ensure the implementation of the Environmental Guidelines for Poultry Farms.

APPENDIX

Appendix-I: Survey report on Antibiotic consumption in poultry

A mini survey was conducted to understand the antibiotics usage, source of antibiotics, purpose of antibiotics usage, decision of dosage, preventative measures and knowledge of the AMR amongst the poultry (broiler) farmers to supplement the research. The survey was conducted in offline mode in some of the poultry (broiler) farmers as well as veterinarians Maharashtra, Odisha, Haryana and West Bengal. Total 25 farmers responded and actively participated in the questioners based on the Antibiotics usage in the poultry. This information is crucial for consumers, policymakers, and stakeholders to understand the scale of antibiotic usage and its potential consequences.

Offline survey

The survey results have highlighted several gaps and areas of improvement for antibiotic management within broiler farming, some of which are provided below:

- The majority of the feed bags available at the farms did not provide information regarding their ingredient list.
- Many farmers are not aware of the risks of antibiotic resistance or the importance of using antibiotics responsibly. This can lead to overuse and misuse of antibiotics.
- Some farmers admitted to administering antibiotics without seeking professional veterinary advice. This points to the necessity of fostering stronger partnerships between farmers and veterinarians, where the veterinarians can guide prudent antibiotic usage, taking into account factors such as dosages, treatment regimens, and withdrawal periods.
- Limited awareness of withdrawal periods among some independent broiler farmers, which can lead to antibiotic residues in poultry products beyond permissible levels, potentially posing risks to consumers and contributing to antibiotic resistance.
- Conventional use of poultry manure in agriculture, gardening, and fish farming is an example of the multifaceted impact of the poultry industry. It is imperative to ensure that these practices remain sustainable and do not contribute to the spread of antibiotic residues or resistance.
- Insufficient biosecurity measures, inadequate farm management practices, and non-compliance with the Environmental Guidelines for Poultry Farms by CPCB were observed.

The preliminary survey indicated that the commonly used antibiotics in the broiler farms were:

- Ceftriaxone
- Levofloxacin
- Azithromycin
- Neomycin & Doxycycline
- Ciprofloxacin
- Lincomycin + Neomycin
- Cephalexin
- Cloxacillin

In addition to the broiler farmers, a few veterinarians associated with the poultry farms were also surveyed. The major findings of the veterinarian survey were:

- All the veterinarians we surveyed used antibiotics during infections.
- Many also prescribed antibiotics for preventive measure.
- Most of the vets have observed resistance in the broilers.
- All were aware of concerns related to antibiotics and AMR.
- Most of the contract farms consult line supervisors (instead of veterinarians) associated with the feed companies for antibiotic usage and other management aspects.

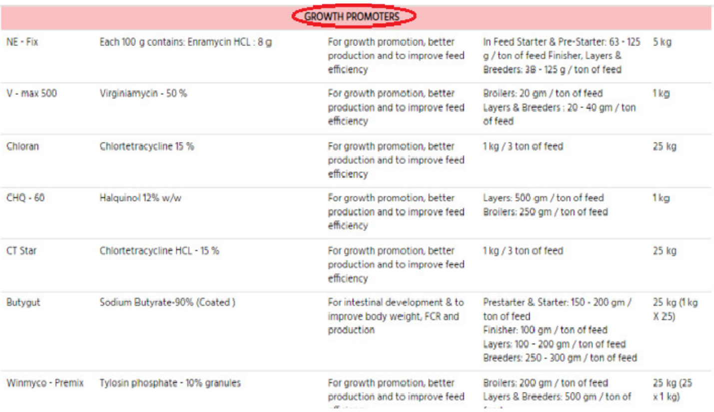
Online survey

Toxics Link also conducted an online survey to determine the feed products marketed and sold as antibiotic growth promoters. The online survey indicated that several feed and drug companies are actively selling antibiotic growth promoters as feed supplements and feed premixes. These products commonly contain Amoxicillin, Tylosin-, Chlortetracycline, Enrofloxacin, Ciprofloxacin, and Doxycycline. The screenshots of these feed company websites are given below.

Although, the use of antibiotics with systemic action is not recommended to be used as growth promoters in poultry feed, amoxicillin, doxycycline, ciprofloxacin, enrofloxacin, etc. are still used in feeds. The survey also revealed a notable observation. Despite the prohibition of the sale, manufacture and distribution of colistin and its formulations in food-producing animals, poultry, aqua farming and animal feed supplements by India in 2019, feed products containing colistin sulphate are still available.

Table 3: Antibiotics in different feeds of the Poultry sector

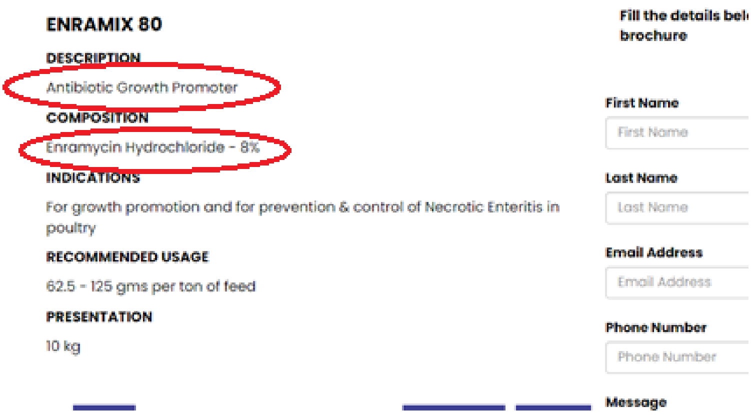
Sr. No.	Local name of poultry feed	Name of Antibiotics	Use
1	Amopremix	Amoxycillin (As Amoxycillin trihydrate) 100gm	Used against gram-positive and gram-negative bacteria, intense bactericidal agent
2	NE – Fix V - max 500 Chloran CHQ – 60 CT Star Butygut Winmyco – Premix Frankolin	Each 100 g contains: Enramycin HCl: 8 g Virginiamycin - 50 % Chlortetracycline 15 % Halquinol 12% w/w Chlortetracycline HCL - 15 % Sodium Butyrate-90% (Coated) 7. Tylosin phosphate - 10% granules 8.Tiamulin Fumerate: 10 %	Growth promoters
3	ENRAMIX 80	Enramycin Hydrochloride - 8%	Antibiotic Growth Promoter
4	BAMBERCIN 40	Bambermycin 4 %	Antibiotic Growth Promoter
5	Colinex Powder	Colistin (As sulphate) – 100mg	Growth promoter, antibacterial
6	CIPRO KBS	Ciprofloxacin – 1000 mg as Ciprofloxacin HC	For the prevention and treatment of CRD, CCRD, Coli bacillosis, Pulloram & fowl/ Typhoid, Staphylococcal infection, Coryza, Secondary bacterial infections of viral outbreaks.
7	Colimex	Colistin sulphate	Poultry feed



GROWTH PROMOTERS

NE - Fix	Each 100 g contains: Enramycin HCL : 8 g	For growth promotion, better production and to improve feed efficiency	In Feed Starter & Pre-Starter: 63 - 125 g / ton of feed Finisher, Layers & Breeders: 38 - 125 g / ton of feed	5 kg
V - max 500	Virginiamycin - 50 %	For growth promotion, better production and to improve feed efficiency	Broilers: 20 gm / ton of feed Layers & Breeders : 20 - 40 gm / ton of feed	1 kg
Chloran	Chlortetracycline 15 %	For growth promotion, better production and to improve feed efficiency	1 kg / 3 ton of feed	25 kg
CHQ - 60	Halquinol 12% w/w	For growth promotion, better production and to improve feed efficiency	Layers: 500 gm / ton of feed Broilers: 250 gm / ton of feed	1 kg
CT Star	Chlortetracycline HCL - 15 %	For growth promotion, better production and to improve feed efficiency	1 kg / 3 ton of feed	25 kg
Butygut	Sodium Butyrate-90% (Coated)	For intestinal development & to improve body weight, FCR and production	Prestarter & Starter: 150 - 200 gm / ton of feed Finisher: 100 gm / ton of feed Layers: 100 - 200 gm / ton of feed Breeders: 250 - 300 gm / ton of feed	25 kg (1 kg X 25)
Winmyco - Premix	Tylosin phosphate - 10% granules	For growth promotion, better production and to improve feed	Broilers: 200 gm / ton of feed Layers & Breeders: 500 gm / ton of	25 kg (25 x 1 kg)

Photo 1: Antibiotics in feed as growth promoter



ENRAMIX 80

DESCRIPTION

Antibiotic Growth Promoter

COMPOSITION

Enramycin Hydrochloride - 8%

INDICATIONS

For growth promotion and for prevention & control of Necrotic Enteritis in poultry

RECOMMENDED USAGE

62.5 - 125 gms per ton of feed

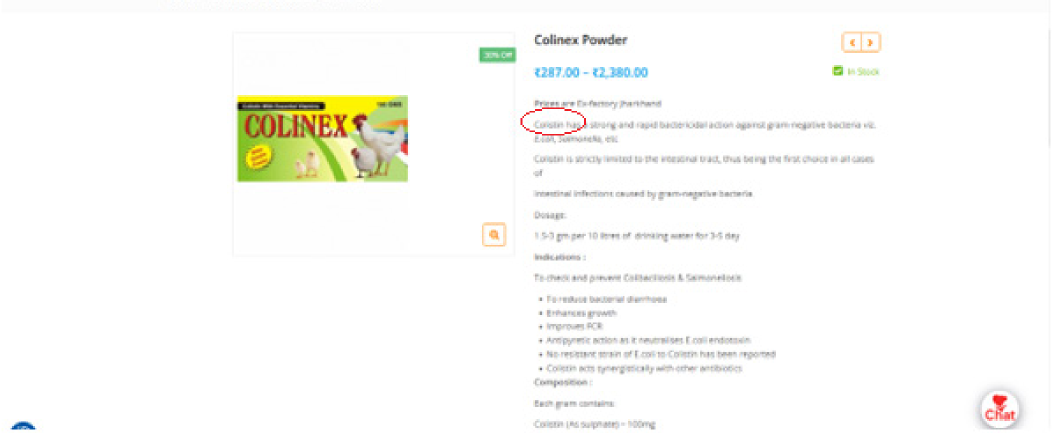
PRESENTATION

10 kg

Photo 2: Antibiotic Enramycin in feed as growth promoter

CIPRO KBS

Category: Poultry Feeds



Colinex Powder

₹287.00 - ₹2,380.00

Prices are Ex-factory Jharkhand

Colistin has a strong and rapid bactericidal action against gram-negative bacteria viz. E.coli, Salmonella, etc.

Colistin is strictly limited to the intestinal tract, thus being the first choice in all cases of

intestinal infections caused by gram-negative bacteria

Dosage:

1.5-3 gm per 10 litres of drinking water for 3-5 day

Indications :

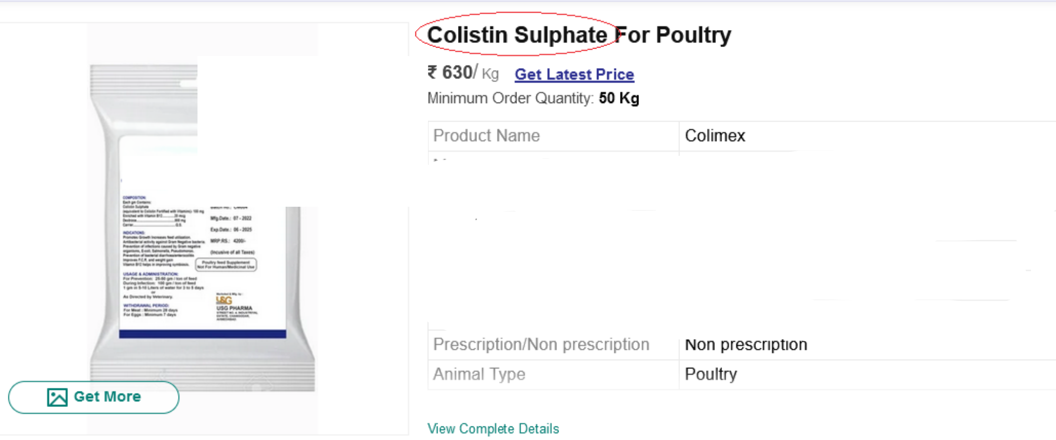
To check and prevent Colibacillosis & Salmonellosis

- To reduce bacterial diarrhoea
- Enhances growth
- Improves FCR
- Antipyretic action as it neutralises E.coli endotoxin
- No resistant strain of E.coli to Colistin has been reported
- Colistin acts synergistically with other antibiotics

Composition :

Each gram contains:

Colistin (As sulphate) - 100mg



Colistin Sulphate For Poultry

₹ 630/ Kg Get Latest Price

Minimum Order Quantity: **50 Kg**

Product Name	Colimex
Prescription/Non prescription	Non prescription
Animal Type	Poultry

View Complete Details

Photo 4: Banned antibiotic Colistin in feed as growth promoter

Appendix-II: Supplementary data

(I) The Comprehensive Antibiotic Resistance Database

The Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca/) is a primary source for reference DNA and protein sequences, detection models, and bioinformatics tools on the molecular basis of bacterial antimicrobial resistance.[82] The Antibiotic Resistance Ontology (ARO) is the primary ontology in CARD as it includes detailed descriptions of the molecular basis for antibiotic resistance, encompassing known AMR determinants (i.e., acquired resistance genes, resistant mutations of housekeeping genes, efflux overexpression, etc.), drug targets, antibiotic molecules and drug classes, and the molecular mechanisms of resistance.

(II) Gene Prediction Statistics

Sample Name	Number of genes	Average gene length (bp)	Length of the longest gene (bp)	Length of the shortest gene (bp)
C4	697,067	594	16,119	60
C5	441,596	519	10,560	60
C6	359,728	631	11,976	60
C7	189,497	504	11,400	60
VD1	280,830	615	10,560	60
VD2	266,869	622	10,662	60
VD3	292,294	618	9,315	60
VD4	279,955	639	9,435	60
VD5	310,711	616	16,221	60
VD6	419,154	632	38,112	60
VD7	91,839	637	19,866	60

(III) Kingdom level abundance statistics of 11 samples

Sample Name	Archaea	Bacteria	Eukaryota	Viruses	Unclassified
C4	0.05	70.78	0.07	0.42	28.68
C5	0.4	79.02	0.11	0.68	19.79
C6	0.04	79.7	0.35	0.14	19.77
C7	0.15	82.95	0.19	1.65	15.06
VD1	0.02	82.29	0.04	0.67	16.98
VD2	0.01	82.56	0.03	0.95	16.45

VD3	0.09	84.63	0.08	1.01	14.19
VD4	0.01	82.69	0.03	0.4	16.87
VD5	0.02	82.71	0.04	0.03	17.2
VD6	0.05	77.07	0.19	0.04	22.65
VD7	0	92.19	0.22	0.11	7.48

(IV) Summary of AMR study

SL. No.	Sample Name	CARD annotated gene counts
1	C4	7,914
2	C5	5,017
3	C6	4,554
4	C7	2,256
5	VD1	3,739
6	VD2	3,546
7	VD3	3,846
8	VD4	3,810
9	VD5	3,711
10	VD6	5,354
11	VD7	1,592

(V) Observations from the samples

Samples	Most abundant organism at species level
C4	Ruania albidiflava
C5	Luteimonas sp
C6	Pedobacter indicus
C7	Virgibacillus ihumii
VD1	Ruania sp.
VD2	Dietzia timorensis
VD3	Brevibacterium senegalense
VD4	Ruania albidiflava
VD5	Stanieria cyanospaera

Endnotes

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