

Antibiotic use in poultry production; a cross-sectional study in a selected district in Sri Lanka

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ABSTRACT

Introduction: Rampant use of antibiotics in animal husbandry contributes significantly to the development and spread of antibiotic resistance. This study determines the antibiotic activity in poultry feed and assesses the knowledge on feeding practices and their potential effects on antimicrobial resistance among poultry farm owners.

Methods: Poultry feed available for sale in shops and ready to serve poultry feed and water samples from selected farms in Kegalle District, Sri Lanka were tested for the antibiotic activity using the agar well diffusion method against three control organisms; *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 and the zone of inhibition around each well was measured. All farm owners were interviewed and basic details regarding the farm such as area, number of animals, water source and the awareness of farm owners regarding antibiotic use in poultry feeds and water source were obtained.

Results: No antibiotic activity was detected in commercially available poultry feed in selected shops (5/5) and ready to serve poultry feed in selected farms (18/18). An antibacterial effect was detected in water samples collected from 33.33% (6/18) of farms. Seventy-eight percent (14/18) of farm owners declared that they administer different types of additives containing antibiotics along with drinking water served to animals. Forty-four percent of farm owners believe that usage of antibiotics in poultry is not a good practice and harmful for human health while a similar percentage claim that it is a favorable practice as it benefits them economically. None of the farm owners were aware that the use of antibiotics in poultry can contribute to the increasing antibiotic resistance in the community.

Conclusion: Commercially available poultry feeds in shops and ready to serve poultry feeds in farms did not demonstrate any antibiotic activity. However, antibiotic activity was detected in drinking water served to animals due to incorporation of antibiotic containing additives beyond the prevailing guidelines. The awareness of farm owners on antibiotic use in poultry and its effects on the increase of antibiotic resistance in the community is unsatisfactory.

Key words: *Antimicrobial resistance, antibiotic activity, food additives, poultry feed.*

Introduction

Poultry is the main livestock species in food production in the world as well as in Sri Lanka. Use of antibiotics as feed additive has become one of the key practices in modern poultry production as it supports growth promotion in animals and helps to treat and prevent infections in poultry. However, it contributes to the development of antibiotic resistance in both animals and humans.

More than 60% of all antibiotics produced worldwide are employed during animal production for both therapeutic and non-therapeutic purposes (1). In the United States, approximately 80% of total antibiotics consumed are used in the livestock sector (2). According to the US Food and Drug Administration, 6.1 million kilograms of medically important antibiotics were sold to US animal producers in 2019, of which 3% was being used for chicken (3).

Furthermore, a considerable portion of the administered antibiotic (17%-90%) is passed to the environment as the original compound or its toxic metabolites directly through urine and faeces. The remnants of poultry feed also contribute to environmental contamination. The emergence of antibiotic resistance genes has been reported around the world due to the misuse of these drugs in animal production (4). The antimicrobial usage in animal husbandry is projected to increase by 67%, and that of poultry by 129% by 2030 (5).

In Europe, salmonella bacteria from poultry and swine showed resistance levels reaching over 80 percent to tetracyclines, sulfonamides and ampicillin. Such data in most of the world are sparse. A study done in the Kandy district, Sri Lanka has identified some bacteria, resistant towards the tetracyclines and enrofloxacin in poultry litter and agricultural soils (6).

The World Health Organization (WHO) has called all nations to develop national strategies to minimize this serious threat of antimicrobial resistance. They have recommended to have an overall reduction in the use of all classes of medically important antimicrobials in food animals and a complete restriction of the use of antibiotics for growth promotion and for the prevention of infectious diseases. Furthermore, WHO suggests that antimicrobial agents that are categorized as

critically significant for humans should not be used to treat or control clinically diagnosed infections in farm animals (7). Sweden is known to be the first country in the world to ban the use of antimicrobial agents for growth promotion in 1986 and prophylaxis in 1988 (1). In 2017, FDA banned the use of antibiotics as growth promoters (8). At present, the only recommended growth promoters that can be used in Sri Lankan poultry industry are Avoparcin, Carbadox, and Flavophospholipol (9).

The Ministry of Health in Sri Lanka has formulated "A national strategic plan for combating antimicrobial resistance in Sri Lanka 2017 - 2022" to support global efforts to address antimicrobial resistance. Specific objective 4.3 under the 4th strategy of this will ensure the prudent use of antimicrobial agents in animals and in agriculture. Although provisions under Animal Disease Act (No. 59 of 1992) and Animal Feed Act (15 of 1986) are currently available to regulate the importation, manufacturing and distribution of antimicrobials in the veterinary sector; several gaps have been identified to fulfil the regulatory mechanism that needs to ensure the prudent use. The implementation of existing regulation is hampered due to limited resources. It is planned to establish a monitoring and evaluation system to ensure rational use of antimicrobials in animal husbandry. (10)

Therefore, the emergence of antibiotic resistance is a global challenge in which antibiotic usage in animal husbandry plays an important role. Data on antibiotic consumption in livestock is limited worldwide. Poultry farms occupy a dominant place in the livestock sector in Sri Lanka due to high consumption of chicken meat and eggs. As a result, poultry farms in Sri Lanka may be contributing vastly for the development of antimicrobial resistance both in human and animals. This study was designed to determine the antibiotic activity in poultry feeds and drinking water for animals used in poultry production in a selected district in Sri Lanka.

Methods

A community-based descriptive cross-sectional study was carried out in poultry farms and poultry feed shops in Kegalle District, Sri Lanka in 2019.

Determining the antibiotic activity in poultry feed available in shops

Two samples from two different types of poultry feed available for sale from five randomly selected shops in Kegalle District were collected into commercially available single-use sterile containers. Samples were stored at 2-8°C in the refrigerator and were transported to the microbiology laboratory in the same condition in cold boxes as soon as possible to determine the antibiotic activity using agar well diffusion method.

Mueller Hinton agar plates were inoculated with a suspension equivalent to MacFarland 0.5 turbidity standard prepared from each control organism; *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853. Five grams (5 g) of each food sample was mixed with 25 ml of sterile distilled water using a vortex mixture. Fifty microlitre (50 µl) each from the suspensions of food samples from one shop (in triplicate), positive control (gentamicin), and negative control (sterile distilled water) were placed in each well with a diameter of 6 mm. All plates were incubated at 35°C, 18 - 24 hours and the zone of inhibition around each well was measured in millimeters.

Determining the antibiotic activity of ready to serve poultry feed and water which are in poultry farms

Eighteen poultry farms were selected from the Kegalle District based on the sample size calculation using the formula for the descriptive study (11) and the prevalence of antibiotic resistance in poultry feed used in poultry production in Galle taken as 25% (12).

Farm owners of all eighteen farms were interviewed by the principal investigator to obtain details such as the area of the farm, number of animals, feeding frequency and amount, water source, using additives containing antibiotics to water and poultry feed, and awareness of farm owners regarding antibiotic use in poultry.

Three samples of poultry feed and three water samples that are ready to serve were collected from different places of each farm. A sample from each

available additive used along with food or water was obtained. Sample collection, temporary storage, transport and testing follow the same procedure mentioned under testing of poultry feed from shops.

Five grams (5 g) of each food sample was mixed with 25 ml of sterile distilled water using a vortex mixture to prepare a suspension. From a collected water sample, 20 ml was centrifuged to separate the last 5 ml of the deposit. When the additives were available, the analysis was done from the neat sample (original concentrations) and at diluted concentrations used to feed poultry. All these samples were tested in triplicate for antibiotic activity using the agar well diffusion method.

Ethical approval was obtained from the Ethics Review Committee of the Faculty of Allied Health Sciences, University of Ruhuna, Galle and permission was obtained from the local authority.

Results

Antibiotic activity of poultry feed available in shops

Two types of poultry feed were available at the five selected shops in Kegalle District, one for chicks and the other for adult animals. All the tested samples did not show any antibiotic activity by the test performed against three standard organisms.

Antibiotic activity of ready to serve poultry feed in poultry farms

Only chickens were raised in all the selected 18 farms. The size of the farm varies from 100 - 1500 m² (mean 672 m² (SD 362.7), median 750m²) and the number of animals in the herd ranged from 100 - 3500 (mean 1422 (SD 1133.6), median 1100). Commercially available food is being used to feed the poultry and it is served approximately 10 g of poultry feeds per animal, two times a day. All samples of poultry feed that are ready to serve did not show any antibiotic activity by the tests performed against three standard organisms.

Determining the antibiotic activity of ready to serve water in poultry farms

In most of the farms (14/18, 78%) well water is being used as the water source and the rest use pipe born water from the water supply board. Same proportion of farms (14/18, 78%) declare that they incorporate one or more additives to the water being served to

animals. Of the farms where additives are added to drinking water, antibacterial activity was detected in water samples collected from 6 farms (6/14, 42.9%). Among these, water samples from 5 farms show anti-staphylococcal activity (Figure 1), 3 farms show anti-*E. coli* activity (Figure 2) and 2 farms show antipseudomonal activity (Figure 3).

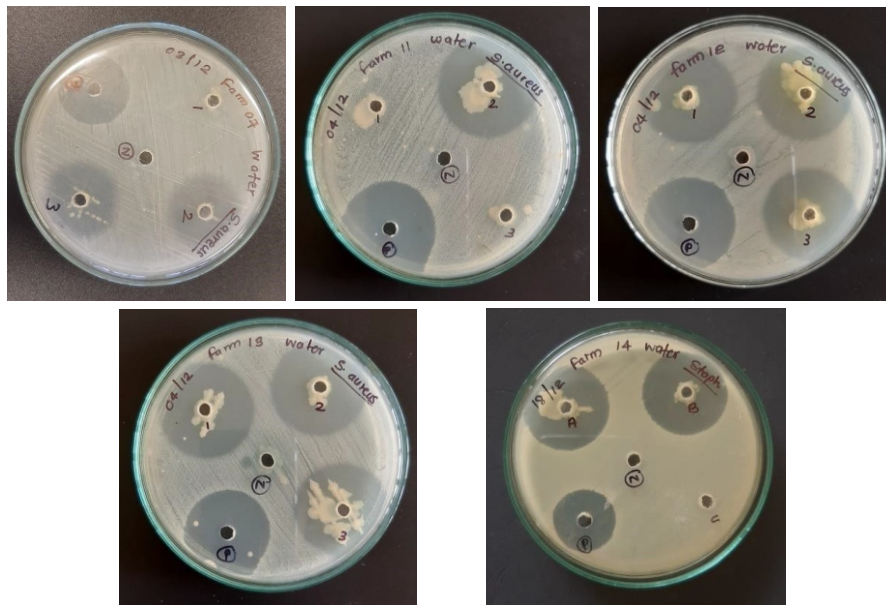


Figure 1: Antibiotic activity of water samples collected from farm No. 7, 11, 12, 13 and 14 against *Staphylococcus aureus* ATCC25923



Figure 2: Antibiotic activity of water samples collected from farm No. 7, 14 and 15 against *Escherichia coli* ATCC25922

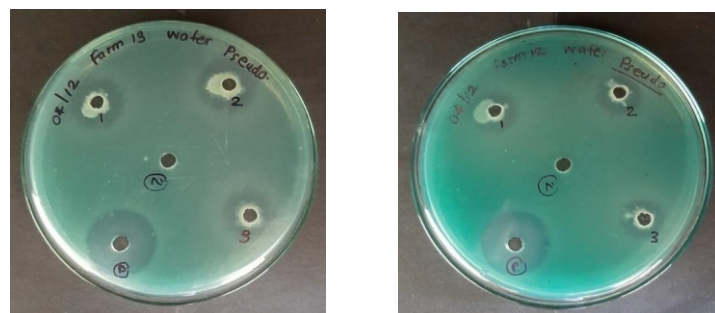


Figure 3: Antibiotic activity of water samples collected from farm No. 12 & 13 against *Pseudomonas aeruginosa* ATCC27853

Antibiotic activity of additives used along with water

The study has identified 8 different types of additives used by the farm owners that were incorporated into water to be served to the poultry (Table 1). Two of the additives (2/8) did not show any antibacterial activity. Rest of the samples (6/8) demonstrated antibacterial activity in the ready to serve concentrations of the additive used to incorporate into water served to poultry (Table 2). Out of them, comparatively higher antibiotic activity than the positive control was demonstrated by the additive number 2, 5, 6 and 7

against *Staphylococcus aureus* ATCC25923 and by the additive number 2, 5 and 7 against *Escherichia coli* ATCC25922.

Neat concentrations of only 4 additives were produced by farm owners, of which 3 has demonstrated antibacterial activity to the neat concentration. Among them the zone of inhibition detected in additives number 1 and 4 were more or less equal to the positive control indicating an equal antibiotic activity as the positive control. But zones of inhibition detected in additive number 2 was more than two times that of the positive control indicating higher antibiotic activity.

Table 1: Description of additives used to incorporate into water served to poultry

Additive number	Content	Remarks
1	A liquid complementary feed composed of carbonic acids and secondary plant compounds that shows some antibiotic effect	Used to improve the feed intake and the body weight gain (a growth promoter). Destroy bacterial pathogens in gastrointestinal tract.
2	100mg of Enrofloxacin (a synthetic fluoroquinolone antimicrobial agent) per 1ml	Used to treat Colibacillosis, Salmonellosis and infections caused by <i>Mycoplasma</i> species.
3	Not declared	Used to increase the body weight and to protect the farm from bacterial infections. Controls the bad odour of cages when sprayed on the cage floor.
4	100 mg of Vitamin E and 0.5 mg of Selenium per 1ml	Used to fight muscular dystrophy, build a better immune system and enhance egg production.
5	200 g of Sulfamethazine and 40 g of Trimethoprim per 1kg	An antibiotic used to treat infections caused by Gram positive and Gram negative bacteria including <i>E. coli</i> , Salmonella and Pasteurella.
6	Tylosin (100% w/w) (a macrolide antibiotic)	Treatment of chronic respiratory diseases and necrotic enteritis caused by <i>Mycoplasma</i> species and <i>Clostridium perfringens</i> respectively.
7	Neomycin and Oxytetracycline	Used to control gastrointestinal and <i>Mycoplasma</i> infections.
8	Not declared	Use not declared

Table 2: Zones of inhibition measured in millimeters against three standard organisms against the neat concentration and the ready to serve concentration of additives

Additive number	<i>S. aureus</i>		<i>E. coli</i>		<i>P. aeruginosa</i>	
	Neat	Ready to serve	Neat	Ready to serve	Neat	Ready to serve
01	29	0	25	0	23	0
02	46	33	54	38	48	21
03	0	0	0	0	0	0
04	0	0	25	0	0	0
05	<i>N</i>	42	<i>N</i>	32	<i>N</i>	0
06	<i>N</i>	32	<i>N</i>	0	<i>N</i>	0
07	<i>N</i>	38	<i>N</i>	27	<i>N</i>	16
08	<i>N</i>	0	<i>N</i>	0	<i>N</i>	0
Negative control	0	0	0	0	0	0
Positive control	23 - 25	23 - 30	21 - 24	21 - 22	17 - 22	17 - 21

N - Not available for testing

Negative controls - zone of inhibition in all tests were zero

Positive controls - zone of inhibition given as a range as each additive was tested separately

Neat concentration - original concentration

Information gathered from poultry farm owners

All farms perform a complete cleaning process once in 30 - 45 days after one whole batch of animals are sent to slaughter and before purchasing a new batch of chicks. First, Bramble which is applied on the floor is removed completely and the cages are cleaned using a disinfectant and left for 2 - 3 days. Next, slaked lime is applied on the floor and kept for 10-15 days for total decontamination. The outside environment of the cages and containers which are used for serve feed and water are thoroughly cleaned.

All selected farms have not reported any disease for past three months at the time of the visit. All farm owners claim that the farms are visited regularly by a public health inspector. When animals get ill they seek the advice from a veterinary surgeon and in the case when an animal is found dead, they are buried without selling for meat. Chicken stool collected in the farms is sold or being used as a fertilizer.

In most of the farms (15/18) all activities in the farm are carried out by the farm owner and his/ her family members and only in 3 farms there were two

extra workers in each. Safety precautions such as wearing gloves and masks are followed by workers only in 11 farms (61%). Forty-four percent of farm owners (08/18) believe that routine use of antibiotics in poultry is not a good practice and harmful for human health. Similar percentage of farm owners' (44%) declared that routine use of antibiotics in poultry is a favourable practice as it benefits them economically. None of the farm owners who responded (16/16) were aware on the effects of using antibiotics in poultry on increasing antibiotic resistance in the community.

Discussion

Food samples collected from shops in Kegalle district as well as ready to serve food samples collected from farms in Kegalle district did not show any antibacterial action against *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853. This verifies that poultry feed itself may not contain any antibiotic substances and such additives are

not being added to food before it is served to animals at the poultry farms.

But water samples collected from 33% of farms has demonstrated antibacterial effect against one or more of the above standard organisms, emphasizing that antibiotics are given to animals along with served drinking water. This study has identified eight additives being incorporated into drinking water of poultry out of which 62.5% containing antibiotics. The antibiotic activity of the concentration of some additives used in the served water samples in some farms are higher than that the positive control indicating a significantly higher antibiotic concentrations are being fed to poultry.

A study done by the Johns Hopkins University Bloomberg School of Public Health demonstrate the presence of residues of 2-10 antibiotics and 7 other medications in feather meal samples; a type poultry feed made out from feathers of killed poultry. They also showed that these residual concentrations are high enough to select the resistant bacterial strains when tested against *E. coli*. (8)

It is found that high antibiotic residues were present in 40% of samples taken from commercially manufactured poultry feed in the Delhi-National Capital Region of which 17% had multiple antibiotics (2).

Although the WHO has prohibited the application of antibiotics as growth promoters in food animal production sector, Sri Lanka still allows the use of antibiotics; avoparcin, carbadox, and flavophospholipol as growth promoters in poultry sector (9). In routine practice, different commercial products; with different trade names are used in poultry farms as growth promoters. Not only in Sri Lanka but also in India, antibiotics are being used as growth promoters across the country highlighting India as one of the global hotspots for antibacterial resistance (13).

The poultry farms in Kegalle District use several antibiotics namely enrofloxacin by 3 farms, sulfamethazine by 3 farms, trimethoprim by 3 farms, tylosin by 2 farms, neomycin by 4 farms and oxytetracycline by 4 farms with the aim of preventing and treating several bacterial infections. Most of them are used in farms daily or once in a week or monthly. In North America, antibiotics

such as tetracycline, bacitracin, tylosin, salinomycin, virginiamycin and bambarmycin are widely used. Also in the United States, tetracyclines are the most commonly used drug which exceeds two-thirds of the total amount of drugs given to animals (14).

The WHO's list of critically important antibiotics for human health includes ampicillin, amoxicillin, cefadroxil, chlortetracycline, doxycycline, erythromycin, flumequine, gentamicin, vencomycin, oxytetracycline, spiramycin, sulfadiazine and sulfadimethoxine which are restricted to use in the veterinary sector (13). But still, Oxytetracycline is used in 4 farms out of 18 farms (22.2%) in Kegalle district. In contrast, in India 11 such antibiotics were found in the chicken feed (2).

Proper guidelines regarding the types of antibiotics and their minimum and maximum amounts to be used for treating several diseases have not yet been formulated in Sri Lanka. Therefore, several drugs are used for treatment and prophylaxis in farms without considering any limitation due to unrestricted sale and over the counter availability of antibiotics in Sri Lanka. Left over water fed to animals containing antimicrobial residues can contaminate the environment via poultry stools, soil and ground water. This factor may also contribute for the emergence of antibiotic resistant strains in the community.

Conclusions and recommendations

Commercially available poultry feeds in shops and ready to serve in poultry feeds in farms in Kegalle District did not demonstrate any antibacterial activity. But drinking water served to animals in poultry farms in Kegalle District exhibit antibacterial activity due to incorporation of antibiotics containing additives. Antibiotics are used in the poultry sector as growth promoters and for prophylaxis and treatment of infections beyond the prevailing national and international standards. A significant percentage of farm owners claim that usage of antibiotics in poultry is a favourable practice as it benefits them economically. The awareness of farm owners on the contribution antibiotic use in poultry to the development of antibiotic resistance in the community is not satisfactory.

National guidelines should be developed to regulate the use of antibiotics as growth promoters declaring the types of antibiotics with their specific concentrations and the maximum residue limits for antimicrobials in poultry products. The prophylactic and sub-therapeutic use of antibiotics in poultry should be banned. A surveillance system should be implemented to monitor these practices as well as to monitor the antibiotic resistance pattern in livestock. Poultry farmers as well as consumers should be educated on the dangers of antibiotic resistance.

Limitations

Some poultry farm owners did not declare all the types of additives they used. Acts, regulations and guidelines regarding the usage of antibiotics in the poultry sector in Sri Lanka were very difficult to find as those data were not freely available.

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