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A Cross Sectional Study on Use of Antibiotic Growth Promoters and Status of Antimicrobial Resistance in Intestinal Non-fastidious Aerobic Bacterial Microflora of Broilers in Vindhya Region of Madhya Pradesh

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Abstract

The usage of antibiotic growth promoters (AGPs) in poultry farming is debatable and use is regulated in some countries including India as their use has raised the concern of positive selection of antimicrobial resistance (AMR) genes in bacteria. With this concern a cross sectional study was carried out to know the uses of AGP among broiler in Vindhya region of Madhya Pradesh by questionnaire survey and identify the antimicrobial resistance (AMR) in four non-fastidious aerobic bacteria of intestine in broilers. A total of 100 broiler farms were surveyed and 300 intestinal samples were collected and processed for isolation and identification of *Staphylococcus, Pseudomonas, E. coli* and *Salmonella* spp. by cultural and biochemical methods and antibiotic susceptibility test. Statistical analysis was carried out by log linear analysis to detect the significance of association between these categorical variables. On questionnaire survey 54% (54/100) responded about usage of antibiotics in their farm, 42 % did not respond and remaining 4% did not use any antimicrobials. One of the five antibiotics including enrofloxacin, cephalexin, levofloxacin, tylosin and azithromycin is being used by the farmers irregularly in feed or drinking water. Antibiotic sensitivity test indicated that all the isolated bacteria were multi drug resistant and a variable resistance and senstivity was detected for the antimicrobials of critical clinical importance in human and animals. Antibiotic sensitivity pattern for the antibiotics being used by the farmers of study region showed highest senstivity to levofloxacin and highest resistance to tylosin and azithromycin among the isolated bacteria.

Keywords: Antimicrobial resistance, Sensitivity, Bacteria, Antibiotic growth promoter

Introduction:

Poultry production is most widespread and one of the rapidly growing food industries. In India broiler poultry farming is well adopted as one of the most profitable animal food production business for small scale as well as large industrial production system. Because of low capital requirement and fast return as slaughter weight of broiler can be attained at the age of 6-8 weeks, broiler production is a very well adopted by small scale producer. In poultry production system two major concerns are high feed conversion ratio and prevention of disease spread in the flock. The use of antimicrobial growth promoters (AGPs) in animal food production system was introduced in 1940s as a sustainable solution to address the increased demand for food. As the promotion of intensive farming in animals sector the use of AGP became more popular (Founou et al., 2016). AGPs are antibiotics which are given to the livestock in feed or drinking water with the objective to increase feed efficiency for better growth and preventing the bird from diseases (Boamah et al., 2016; Miyakawa et al*.,* 2024). Antimicrobial growth promoters (AGPs) are commonly used in broiler production for rapid attainment of weight and as prophylactic measure of disease spread control. AGPs are generally used as the sub therapeutic doses for a longer duration, that raised the concern of risk of selection and spread of resistant genes in bacteria in animals and their dissemination to humans or the environment (Mehdi et al., 2018; Untari et al., 2021). About 60% of all produced antibiotics used in animal production system for both therapeutic and non-therapeutic purposes (Cully, 2014). In many developing countries the intensification of farming leads to increase in extent of usage of antibiotics in food animals. Many of the essential antibiotics intended for both human and animal usage are employed in poultry production in many countries. Concern of indiscriminate use of AGP in animal food production system may severely affect the public health in terms of prominent contributing factor to the development and spread of antibiotic resistance that may results in treatment failures (Michael, et al*.,* 2015). However, the increasing concern for antimicrobial resistance and consumer preference for antibiotic-free poultry products has led many countries to ban or restrict the use of AGP. Currently, in the United States, medically important antibiotics used in feed and water for

food-producing animals are only allowed with the stipulations of the Veterinary Feed Directive (Food and Drug Administration, 2021, 2023; Fonseca et al., 2024). Some countries particularly European Union (European Commission, 2005) have the guidelines for restricted use of antibiotics as growth promoters however many countries particularly developing countries do not have specific guidelines. In India the consumption of antibiotics is estimated about 3% of global antibiotic consumptions in which 82 % increase is estimated by 2030 (Van Boeckel et al*.,* 2015). Comprehensive ban on the use of AGP in production system has been issued by food safety and standard authority of India recently in 2024, however, monitoring this restriction at farm level is challenging task.

There is a paucity of data about the type and amount of AGP used in poultry sector and their effects on their gene pool of the bacteria. A limited effort has been made to evaluate the comparative effectiveness of commonly used AGPs in modulating gut microbiota of chickens. Further, understanding the effects of AGPs on the diversity and community structure of the gut microbiome is important for devising strategies for developing alternatives to AGPs for improving the chicken gut microbiome. The ongoing concern about developing AMR need to identify the pattern of AMR in species particularly poultry microflora so that clear insight can be outlined. Considering all these facts in light this study was designed to identify the antibiotic sensitivity profile of four non-fastidious aerobic/facultatively anaerobic bacteria from intestine and to conduct the questionnaire survey to find out the usage of antibiotic growth promoter and other antibiotics in poultry or knowledge level of farmers about AMR.

Materials and Methods:

Sampling

One hundred randomly selected broiler farms from the 25 block of Rewa and Shadol division were surveyed and total of 300 broiler intestinal samples were collected freshly from the poultry shops of these areas [the source of bird of these shop could not be stipulated and the poultry farm producer (surveyed) and supplier of birds to these shop may not be same]. Questionnaire survey was carried out by taking verbal consent of the farmers and observing the farm practices. The questionnaire included questions about the housing, management and uses of antibiotics in broiler production system and their knowledge level about the antibiotics and AMR. All samples were collected aseptically into sterile containers, and then transported with cooler boxes immediately to the laboratory of the Department of Veterinary Public Health & Epidemiology, College of Veterinary Science & A.H, Rewa. Samples were processed at earliest, preferably within four to six hours of collection for isolation and identification of *Staphylococcus, Pseudomonas, E. coli* and *Salmonella* by cultural and biochemical methods.

Phenotypic characterization of isolates

One gm of intestine samples with its content were triturated with the help of pestel and mortar, and inoculated in 9 ml of nutrient broth and MacConkey broth separately and incubated at 37 °C for 24 hrs. After incubation a loop full of culture from nutrient broth was inoculated on Mannitol salt agar and Cetrimide agar for selective growth of *Staphylococcus* and *Pseudomonas,* respectively. A loopfull from MacConkey broth was inoculated on the MacConkey agar, EMB agar, Hektoen enteric agar (HEA), and nutrient agar (Himedia) for the selective growth of *E coli* and *Salmonella* spp. Isolated cultures from all the samples were characterized and identified by colony characteristics, morphology and different biochemical tests including catalase, oxidase, indole, methyl red, Voges Proskauer, citrate utilization and urease agar, nitrate reduction and sugar fermentation tests.

Antibiotic Sensitivity Profile Testing

Isolated bacteria were detected for antibiotic sensitivity by disc diffusion method. Few colonies of each isolate were inoculated into the brain heart infusion broth. (Himedia) The turbidity of growth of bacteria in BHI was matched with 0.5 McFarland standard tubes. Antibiotic sensitivity test (AST) was carried out on Mueller–Hinton agar (MHA) for 20 antibiotics (Himedia) from 11 classes by following standard protocol of Kirby-Bauer disc diffusion method. The plates were incubated at 37°C for 24 hr and zone of inhibition was recorded for each antibiotic for every isolate. The zones of inhibition obtained for each bacterium was compared with the standards of the Clinical and Laboratory Standards Institute (CLSI, 2020), European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2025), some other research references were used where CLSI standards were not available. Tylosin – *Staphylococcus* (Entorf et al., 2014), *Salmonella* (Hidanah et al., 2022).

Questionnaire survey

A questionnaire survey was also carried on broiler farms as well chicken shops for various managemental, nutritional and health related aspects including biosecurity and use of antibiotic growth promoter or other antibiotics. The questionnaire includes different question related to sanitation and hygienic practices followed by the producers in term of their housing, feeding, vaccination and awareness about antibiotics. To understand the sensitivity of bacterial isolates for the

antibiotics being used by the farmers AST was carried out.

Statistical analysis

Data was analyzed to calculate the positivity of the samples for bacterial isolates, antibiotic resistance percentage for each isolates and use of AGP by farmers. These variables were analyzed by log-linear analysis to detect the significance of interaction by three way analysis using chi-square test to calculate the likelihood ratio and Pearson correlation in SPSS-20 software. The criteria of assessment of association was tested by K-Way and Higher-Order Effects with $P<0.05$ for significant association. The partial association between two of possible interaction between bacteria type, antibiotics type and the resistance pattern was also detected to check the validity of result.

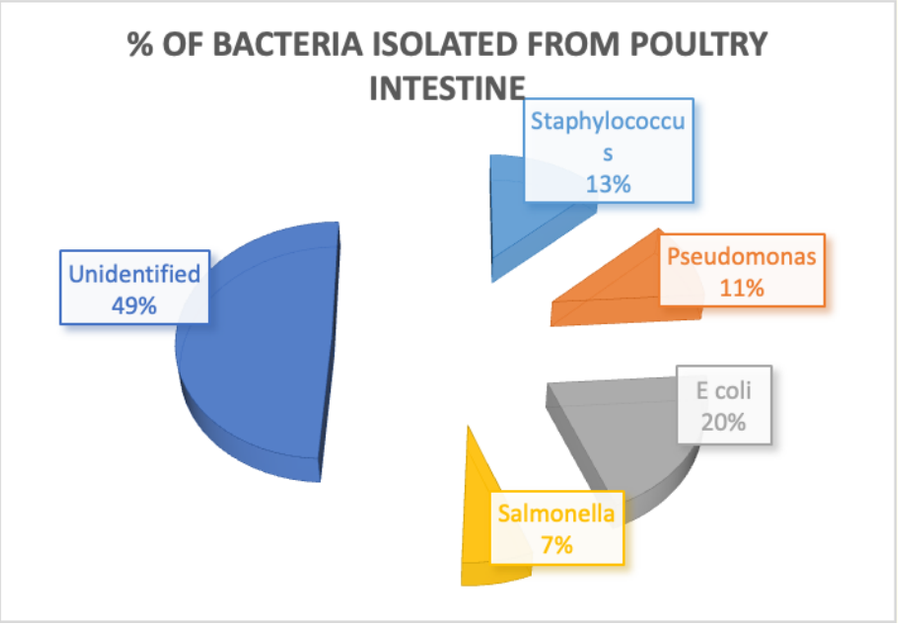

Figure 1: Prevalence of isolated bacteria in broilers

Results and Discussion:

Isolated *Pseudomonas* spp. was resistant to ampicillin, trimethoprim, cefotaxime and ceftazidime. Variable resistance was detected for methicillin 50%, oxacillin 50%, erythromycin 33%, co-trimoxazole 50%, and some cephalosporin; while sensitive for imipenem, streptomycin, vancomycin, gentamicin, ceftriaxone and cepfoperazone. *E. coli* isolates were resistant to methicillin, oxacillin, ampicillin, and tetracycline, however for vancomycin 33%, cefixime 50% variable resistance, while sensitive to imipenem, streptomycin, gentamicin, trimethoprim, erythromycin, co-trimoxazole, ciprofloxacin, doxycycline, chloramphenicol and some cephalosporin. *Salmonella* were resistant to methicillin, oxacillin, ampicillin, and tetracycline, and for erythromycin 33% and co-trimoxazol 50% resistance was noted. Isolates were sensitive for imipenem, streptomycin, gentamycin, trimethoprim, ciprofloxacin, doxycycline and chloramphenicol. It is an alarming situation where bacterial isolates were showing resistance for broad spectrum antibiotics like trimethoprim, tetracycline, vancomycin, cephalosporin and co-trimoxazole and this will create very serious issue in treating certain infections not only in poultry, livestock but in humans too.

On questionnaire survey only 54% (54/100) broiler farmers replied on the question of usage of antibiotics in their farm and 42% didn't respond anything and remaining 4% denied to use any antimicrobials. Pattern of use of AGPs was found to be very irregular in which 24.07% farmers do not follow any regular pattern, 24.07% farmers use antibiotics on alternative day basis, 14.81% uses once in a week, and11.11% use antibiotics on daily basis in feed and water while rest of the poultry farmers did not give satisfactory answer but didn't deny the use of AGPs. All the isolated bacteria have shown a variable sensitivity for the antibiotics being used in the poultry production system as found during questionnaire survey. The antibiotics being used by the farmers include enrofloxacin (35%), levofloxacin (13%), cephalexin (Lixen) (24%), tylosin (20%), and azythromycin (8%). The Antibiotic sensitivity pattern for enrofloxacin,

levofloxacin, Lixen, tylosin and azythromycin of *Salmonella, E. coli, Staphylococcus* and *Pseudomonas* is indicated in the Figure 2.

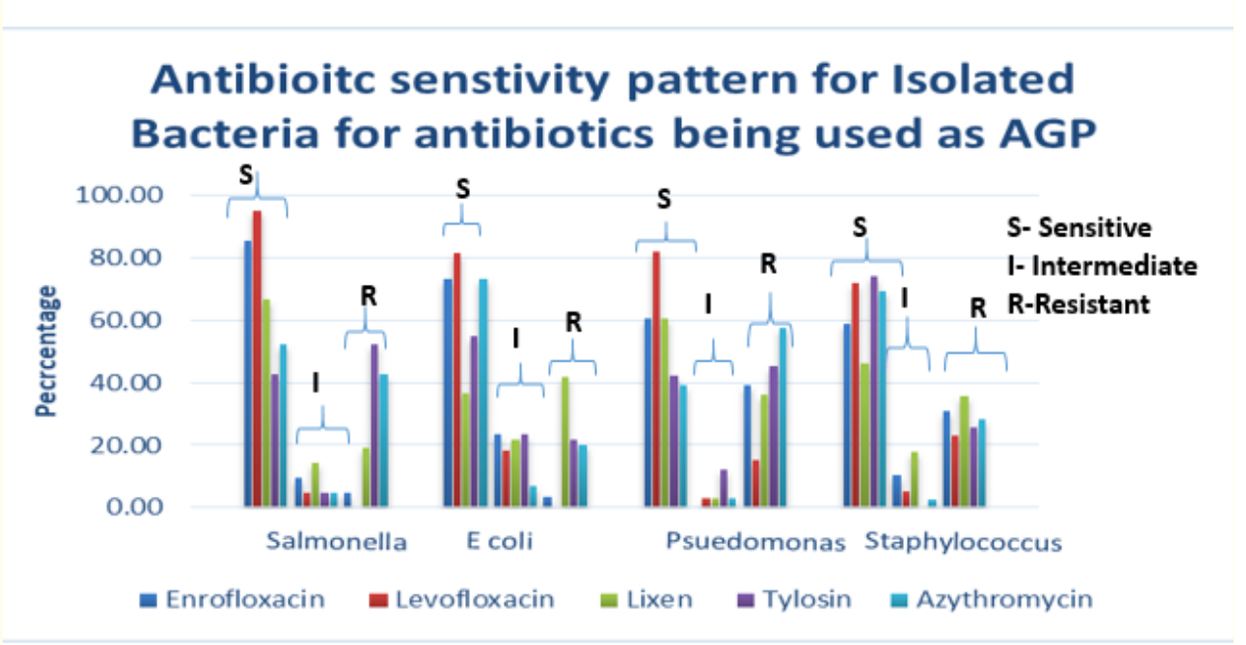

Figure 2: Antibiotic Sensitivity profile of the isolated bacteria

Table 2: Log-linear analysis of detection of significance of interaction among study variables:

K-Way and Higher-Order Effects							
	K	**df**	**Likelihood Ratio**		**Pearson**		Number of Iterations
			Chi-Square	**Significance**	**Chi-Square**	**Significance**	
K-way and Higher Order Effects[a]	1*	59	640.148	0.05	641.572	0.05	0
	2*	50	201.214	0.05	165.478	0.05	2
	3*	24	81.618	0.05	75.930	0.05	4
K-way Effects[b]	1*	9	438.934	0.05	476.094	0.05	0
	2*	26	119.596	0.05	89.548	0.05	0
	3*	24	81.618	0.05	75.930	0.05	0
a. Tests that k-way and higher order effects are zero.							
b. Tests that k-way effects are zero.							

1* = Main Effect, 2* = Two way interaction effect, 3* = Three way interaction effect

Partial Associations				
Effect	**Df**	**Partial Chi-Square**	**Significance**	**Number of Iterations**
Bacteria*Antibiotic	12	3.525	0.991	2
Bacteria*BacterialType	6	50.181	0.05	2
Antibiotic*BacterialType	8	69.083	0.05	2
Bacteria	3	99.482	0.05	2
Antibiotic	4	.409	0.982	2
BacterialType	2	339.042	0.05	2

In this study four bacterial isolates including *E. coli, Salmonella, Staphylococcus* and *Pseudomonas* were detected in broiler intestinal samples (Figure 1). These isolates may cause serious disease in chicken along with possible transmission to human through meat, direct contact, and aerosol during occupational exposure. Food borne transmission and occupational exposure to poultry retail products may transmit *P. aeruginosa* to humans. This transmission not only causes the treatment difficult in clinical infection cases due to development of antibiotic resistance but there is a possible risk of transfer of antibiotic-resistant genes among poultry and human pathogens (Marouf et al., 2023). *P. aeruginosa* have been reported with the prevalence of 12 % in healthy chicks and 30% diseased ones and 8 to 21 % from broiler chicken flock earlier (Abd El-Ghany, 2021). *Staphylococcus* is considered as an opportunistic pathogen that may cause a set of infections and have ability to colonize in various livestock animals including sheep, goats, cows, and poultry. It has been known to be a major cause of lameness in the poultry and reported to be found in the intestinal tracts of birds, including chickens, waterfowl and turkeys (Rao et al., 2023). Some strain of *E. coli* serovar O2 and O78 of avian origin are highly pathogenic and causes avian colibacillosis in poultry and is generally referred as Avian pathogenic *E. coli* (APEC). In *E. coli* and *Salmonella* the occurrence of resistance for cephalosporin antibiotic group, particularly of third and fourth generation is matter of great concern. In this study, 20-30% resistance for ceftriaxone and cefixime, in *Salmonella* and in *E coli* up 50 % for cefixime while both the isolates were resistant to tetracycline. Avian pathogenic *E. coli* of zoonotic importance are often highly resistant, especially to tetracycline, streptomycin, and sulfonamides (Igbinosa et al., 2014). While clinical resistance is different from resistance in agricultural and environmental bacteria which generally are not pathogens, so the "resistant" term would be either for individual isolate or the whole community of bacteria, and it impart the indirect threat to public health (Ahmad et al., 2021). Substantial number of isolates of detected bacteria had AMR, for WHO's criteria of critically and highly important antibiotics in animal and human clinical disease treatment, indicating a high risk (Abreu et al., 2023).

In this study the antibiotics being used by the farmer include enrofloxacin, levofloxacin, lixen, tylosin and azithromycin. Similar scenario was observed in other developing countries, including Bangladesh, in the animal food production system including poultry production in Ghana, Africa (Annan-Prah et al*.,* 2012). Many antimicrobials, like gentamicin, sulfonamides, erythromycin, ciprofloxacin, enrofloxacin, doxycycline, oxytetracycline, colistin, amoxicillin, and occasionally furazolidone, are widely used in poultry production in Bangladesh, in combination with other antibiotics (Tasmim et al., 2023). Globally most widely used antibiotics are penicillins, tetracyclines, and quinolones particularly with higher usage in countries with meat-heavy diets. In Indian poultry sector, AGP use lack monitoring and as per this study also the famers are not following any specific protocol of antibiotic usage. The farmers are using AGP and other antibiotics not categorized as AGP for preventing their bird from infectious diseases without following appropriate dosing and /or completing the schedule.

In comparison to benefits of AGPs in animals and poultry, their negative effects cannot be outweighed if not used as per the regulations. The presence of their trace amounts in the form of residues in chicken carcasses may also impact on promotion of AMR. The presence of residues of enrofloxacin and ciprofloxacin in various tissues (Liver, kidney, muscles and feathers) for variable interval of time has been reported (Atef et al., 2020; Ringenier et al., 2024; Kharate et al., 2025). One poultry meat sample purchased from Gurgaon a cocktail of three antibiotics was detected (CSE, India). Antibiotics and their bioactive breakdown products are widely considered the primary driver of AMR development (Nhung et al*.,* 2017). Meat producing farms were reported to have twice the rates of antimicrobial resistance as compared to egg producing farms, as well as higher rates of multidrug resistant bacteria (CDDEP, 2017).

Many countries have banned AGP use in feed including European countries, Thailand Sweden, Switzerland and Canada. In India also official ban on AGP with withdrawal periods was way back imposed since 2012. However still the United States (13%), Brazil (9%), China (23%), India (3%), and Germany (3%) are still the top five consumers which uses antimicrobials for animal food production (Van Boeckel et al., 2015).

The viability of antibiotic-free poultry production is questionable because the challenges arise after stoppage of antibiotics, like control and prevention from infectious agents, especially for small scale poultry farmers (Haque et al*.,* 2020). So the uses of AGP and other antibiotics can be restricted and rational usage of antibiotics should be adopted. Usage of antibiotics in-feed should be curtailed step-wise, reducing from a multi AGP usage to a single AGP and cutting down of AGP in different phases. Instead of use of antimicrobial for growth promotion, other gut health promoting compounds like organic acids, probiotics, prebiotics, enzymes etc. for the control and prevention of infection and effective biosecurity measure must be applied in poultry farms (Abd El-Hack et al., 2022). Apart from this, the poultry farmers must be educated and informed about the harmful effect of antibiotics long term usage for the community and public health.

Conclusion:

Poultry production environments represent important reservoirs of antibiotic resistant bacteria that may spread to human populations. Poultry farmers are using to higher group of antibiotics. Ineffective implementation and lack of awareness about the rules and guideline of use of AGP in poultry was observed Vindhya region of Madhya Pradesh, India. There is urgent need to find an option to AGP in the alternative medicine like herbal medicine or homoeopathy as they may be more appropriate in developing immune status gradually and effective antibiotics will be available for clinical disease. Other than this there is the need of finding other option related with nutritional supplement and management factors and practices to improve the growth of birds and prevention of diseases so that AMR development can be minimised. Reducing the use of antibiotics in poultry production system and moving towards an antibiotic-free production, will not only minimize the AMR risk but also enhance the quality of chicken meat been produced, increasing the efficiency of poultry production and profitability.

Conflicts of interest:

The authors declare no conflict of interest. The authors declare no competing financial interests.

Data availability:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contribution:

B.D., A.P.S., S.T. and R.V.S. designed the research. M.D. and A.K.S performed the data analysis. All authors contributed to the writing.

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