Detection of antibacterial activity in chicken meat, eggs, drinking water, animal feed and sewage waste in Tabanan, Bali

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Keywords

antibacterial, resistance, livestock

Abstract

Background: The use of antibiotics that are not in accordance with the indications, doses, and duration can trigger resistance and there is concern that it might leave antibiotic residues in the processed product. Aim of this study was to detect the antibacterial activity of livestock products, namely chicken meat and eggs and the surrounding environment such as drinking water, animal feed and waste disposal. This study was a preliminary study before the establishment of antibiotic wise village, One Health approach for antimicrobial stewardship program.

Methods: This study was a descriptive study with a cross sectional design to determine antibacterial activity, particularly tetracycline in livestock products and the environment. The research samples were taken from 5 groups of farmers in one of the villages in Tabanan, Bali. Bioassay method based on the Kirby Bauer method was used in this study.

Results: From a total of 44 samples, 6 samples showed weak antibiotic tetracycline activity (13.6%), namely in waste disposal (20%) and animal feed (40%). Antibiotic contamination was likely to occur because the animal feed used in this group contains antibiotics with or without the knowledge of the farmers themselves. Disposal waste came from livestock manure that ate the feed or from animal feed that was scattered around the cage.

Conclusion: Samples of livestock meat and eggs did not show antibacterial activity. There were samples that have antibiotic activity but weak and inconsistent, namely in samples of waste disposal and animal feed. This condition cannot necessarily be concluded as antibiotic abuse in livestock however it can be the basis for the importance of providing education regarding antimicrobial resistance. Unless there was indication, antibiotics should not be given to livestock on a daily basis.

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ORIGINAL ARTICLE

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Detection of antibacterial activity in chicken meat, eggs, drinking water, animal feed and sewage waste in Tabanan, Bali

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ABSTRACT

Introduction: The use of antibiotics that are not in accordance with the indications, doses, and duration can trigger resistance and there is concern that it might leave antibiotic residues in the processed product. Aim of this study was to detect the antibacterial activity of livestock products, namely chicken meat and eggs and the surrounding environment such as drinking water, animal feed and waste disposal. This study was a preliminary study before the establishment of antibiotic wise village, One Health approach for antimicrobial stewardship program.
Method: This study was a descriptive study with a cross sectional design to determine antibacterial activity, particularly tetracycline in livestock products and the environment. The research samples were taken from 5 groups of farmers in one of the villages in Tabanan, Bali. Bioassay method based on the Kirby Bauer method was used in this study.
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Conclusion: Samples of livestock meat and eggs did not show antibacterial activity. There were samples that have antibiotic activity but weak and inconsistent, namely in samples of waste disposal and animal feed. This condition cannot necessarily be concluded as antibiotic abuse in livestock however it can be the basis for the importance of providing education regarding antimicrobial resistance. Unless there was indication, antibiotics should not be given to livestock on a daily basis.

Keywords: antibacterial, resistance, livestock.
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INTRODUCTION

Chicken meat is one of the products of livestock origin that has a high consumption rate, because it is easy to obtain, fast growth, and the price is also more affordable compared to large livestock species.[1] The demand for broiler meat tends to increase every year.[2] This condition triggers an imbalance in demand and supply so that producers, in this case farmers, are competing to accelerate production and prevent the occurrence of diseases in livestock that have the potential to fail the harvest. Livestock health conditions and optimization of production can be done by controlling diseases that can attack livestock hence modern animal production practices are associated with regular use of antimicrobials.[2]

Beside being used to treat disease and prevent disease, the use of antibiotics in animal husbandry also aims as a growth promoter. The use of antibiotics as growth promoters or for the treatment and prevention of diseases that are not appropriate, excessive doses, and do not pay attention to the withdrawal time can cause antibiotic residues in muscles and processed products.[3] Based on data from the Indonesia Ministry of Agriculture Survey in 2017, 81.4 percent of farmers use antibiotics in poultry for disease prevention, 30.2 percent use it for treatment and 0.3 percent still use it to accelerate growth.[3]

Besides being harmful to health, antibiotic residues can also affect the environment and economy. For certain combinations of bacteria and antimicrobials, resistance in bacteria from humans was associated with resistance in bacteria from food-producing animals which, in turn, was related to antimicrobial consumption in animals.[4] In the United States, the Animal Health Institute estimates that annually more than 8000 metric tons of these compounds are commonly used in the production of the major livestock species of swine, cattle and poultry. During the 1990s, a ban on the use of antibiotics as growth promoters

was introduced in the European Union (the ban came into effect in 2006) due to concerns about residues in livestock products such as meat, eggs and milk.[5]

The U.S. Department of Agriculture inspected meat, poultry, and egg products and rarely found residues at safe levels. The Centers for Disease Control and Prevention in the U.S. reported that resistant bacteria are increasing and alarming due to the administration of low doses of antibiotics over a long period of time. Because of this, the FDA also began to limit the use of antibiotics as growth promoters. Antibiotics are still allowed to be used as drugs in livestock but must be in accordance with supervision (with certain indication).

Cases of antibiotics residues in poultry products in Indonesia were found in 1993- 2004. Around that time, the range of antibiotic residues found in chicken meat is 8% -70%. This number was indeed concerning. The case antibiotics residues in poultry products found in some areas with different types of antibiotics. With the collaboration of various parties, antibiotics residues case in 2011-2016 in Indonesia was decreased, even some research did not find any antibiotics residues in poultry product however small concentration (4,1% and 4,17%) of tetracycline residue was still found.[6]

The purpose of this study is to determine the antibacterial activity or antibiotic residue contamination cases, particularly tetracycline in livestock and their products such as meat and eggs as well as the surrounding environment such as drinking water, animal feed and waste disposal. The results of this test are expected to increase the awareness of farmers and consumers of the antibiotic contamination in livestock products and environment.

METHODS

Research design

This research is a descriptive study with a cross sectional design to determine the residue of antibiotics in livestock products and the environment. The research was conducted from September 1, 2022 to December 31, 2022 at Universitas Warmadewa. The target population of this study were farm animals. The target population was farm animals in the Bengkel Village, Tabanan. The research samples were chicken meat, chicken eggs, animal feed, drinking water, and waste disposal.

Figure 1. Sample preparation process

Sampling was conducted purposively in a group of farmers in one of the villages in Tabanan. The number of chicken meat samples was taken at the same part of the thigh, and the selection of egg samples was taken by the same method. Sampling was done aseptically. Then the samples were brought to the biomedical laboratory of Universitas Warmadewa, with a cool box to test for the presence of antibiotic residues using a bioassay method based on the Kirby-Bauer method.[6-8] Antibiotic residue testing in this study was carried out by bioassay screening method based on SNI 7424: 2008; BSN, 2008.

Bioassays are performed using the microorganism *Bacillus cereus* to detect active tetracycline compounds.[9,10] The principle of this test is that inhibition of bacterial growth by antibiotics contained in the sample indicates antibiotic residues. Bioassay method on chicken meat to test the content of antibiotic residues in this study was carried out by weighing each chicken meat sample weighed as much as 10 g then added aquabidest. Then homogenized using a homogenizer, then centrifuged at 3000 rpm for 10 minutes. The supernatant was then taken and ready to be used as a test sample solution.[10]

Preparation

Testing for antibiotic residues in chicken meat, eggs, animal feed, animal drinking water and waste disposal using the bioassay screening test method, starting with the preparation of agar media, preparation of culture media, and preparation of standard solutions. The specific bacteria used for the preparation was *B. cereus* ATCC 11778, and antibiotic disks in this study tetracycline as a positive control.[6] Bacterial suspension was made by taking one *ose* of *B. cereus* colonies that have been provided and then put into a test tube that has been filled with 5 mL of 0.9% physiological NaCl. Suspension of *B. cereus* ATCC 11778 with McFarland density/turbidity standard 0.5%.

Sample examination

The first step taken was to make 20 ml of Mueller-Hinton agar media and cool it until it became solid then inoculate the *B. cereus* ATCC 11778 bacterial suspension that had been made according to the above provisions into a petri dish containing agar and then allowed to dry. All paper discs were placed right on the surface of the agar medium, each petri dish containing 4 paper discs with a minimum distance of 2.6 cm from each other. On each paper disk 20 ul solution made from each sample was injected. Tetracycline paper discs were placed as a positive control, and one paper disc containing 20 uL of aquabidest as a negative control.

Petri dishes were then incubated in an incubator at 36°C ± 1°C (37°C is used) for ±18-24 hours to allow maximal growth.[11-13] The results of the antibiotic residue testing method were obtained by observing the inhibition zone formed after the incubation period is complete and then measuring its diameter using a caliper. The inhibition zone was the effect of the presence of antibiotics that inhibit the growth of bacteria around the disc paper, in the form of a clear zone. The diameter of

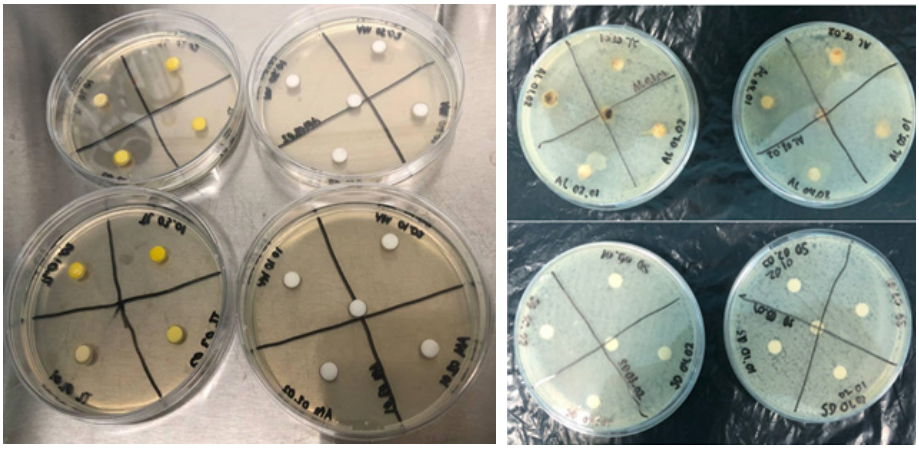

Figure 2. Test for the presence of antibiotic residues using a bioassay method based on the Kirby-Bauer method (left side before incubation, right side after incubation)

the inhibition zone formed indicated the residual concentration of the antibiotic.[10,11] The sample tested positive for residual antibiotics if the zone of inhibition formed at least 2 mm larger than the diameter of the of the disc paper.[10]

RESULTS

In this test, a total of 44 samples from 5 farmer groups were obtained. The distribution is 2 samples from each farmer group for chicken meat, drinking water and waste disposal, 2 egg samples each from 2 farmer groups only (no eggs were found at the time of sampling in 3 other groups). There was no zone of inhibition/ antibacterial activity of tetracycline in the chicken meat samples, in this case the thighs and eggs. Drinking water samples were also clear of tetracycline.

Weak positive results were obtained in sewage and animal feed samples. Positive results in waste sewage samples were found in random samples taken from groups 3 and 4 while in animal feed samples, positive results were obtained from samples of groups 1, 3, 4 and 5 but in farmer groups 1 and 5 the results were inconsistent during the repetition test. From the total number of samples, 6 samples out of 44 samples showed weak antibiotic activity (13.6%), the inhibition zone was 2 mm larger than the diameter of the disc (minimal). In the sewage sample group, there were 20% or 2 out of 10 samples, while in the animal feed sample there were 40% (4 out of 10) samples suspected of antibiotic activity.

DISCUSSION

The result of this study was in line with previous research conducted in Blitar in 2019 which did not find antibiotic content from chicken meat but was found in animal feed.[14] Also, in accordance with the bioassay test results in Selemadeg Timur, Tabanan Bali that revealed all of samples (chicken and duck meat) were negative for tetracycline, aminoglycoside, macrolide, and penicillin antibiotic residues and considered safe for consumption.[9]

A research that took samples from traditional markets in Kendari, Southeast Sulawesi in 2015 showed that 66.6% broiler chicken samples contained residues of tetracycline antibiotics, 100% samples contained macrolides and 60% contained aminoglycosides.[10] Antibiotic residues were not detected in meat and liver except penicillin residues which were found in breast meat, thigh and liver of chicken from Trienggadeng sub district, however as much as 75% (18/24) samples of positive eggs contain penicillin antibiotic residues in one study at Yogyakarta, Indonesia in 2020.[7]

In this study, inhibition zones were found in sewage and animal feed only. The test was carried out from the original sample, not diluted but not extracted so there was still a possibility that there were small amounts of antibiotic residues in other samples but it was also possible that the antibiotic content was below the limit hence it did not cause residues in other samples such as chicken meat and eggs. Antibiotic contamination was likely to occur because the animal feed used in the group contains antibiotics with or without the knowledge of the farmers themselves. Disposal waste was derived from the feces of animals that eat from the feed or animal feed that was scattered in the cage.[6-9,15]

In percentage terms, 4 out of 10 samples of animal feel had positive results but what was concerning was 4 out of 5 farmer groups had at least 1 sample with weak positive results, namely farmer groups 1, 3, 4, and 5. Only samples from farmer group 2 were clean from antibiotic contamination. It was possible that the feed ingredients used contained natural antibiotics (from plants or grains) but at very low levels that did not leave residues in the meat or eggs. The limitation of this study was the qualitative result of antibacterial activity and it was also limited to only one type of antibiotic (tetracycline).[7,8] This study expected to be continued after the socialization to the farmers was done.

CONCLUSION

Meat and egg samples of livestock did not have antibiotic tetracycline residues. There are samples that have antibiotic activity but weak and inconsistent, namely in animal feed samples and sewage samples. This condition cannot necessarily be concluded as the misuse of antibiotics in livestock because there were many factors that allowed this condition to occur. Inappropriate use of antibiotics in livestock should be avoided to prevent resistance and the possibility of residues in livestock products. Future research is expected to be carried out with a larger number of samples and better comprehensive methods, so that the results obtained will be more reliable.

CONFLICT OF INTEREST

The authors declare that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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ETHICAL CLEARANCE

This study has received ethical approval from animal ethic committee of Faculty of Veterinary Medicine, Universitas Udayana with reference letter number B/133/UN14.2.9/PT.01.04/2022 and ethics committee of the Faculty of Medicine and Health Sciences, Universitas Warmadewa with number 306/Unwar/FKIK/EC-KEPK/II/2023.

AUTHOR CONTRIBUTION

NNSB and KKA were involved in concepting the study. MS and NKSY conducted the study. IGARW and IKAIA analyzed the data. MS, AAGI, and PAS constructed the concept, designed, and supervised the manuscript. All authors prepare the manuscript and agree for this final version of manuscript to be submitted to this journal.

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