**Detection of Antibiotic Residues in Chicken Meat and Eggs from Traditional Markets at Yogyakarta City Using Bioassay Method**

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Abstrak

Studi tentang kandungan residu antibiotik dalam makanan yang berasal dari hewan saat ini diperlukan untuk mendukung program kesehatan masyarakat veteriner. Penelitian ini menggambarkan metode *bioassay* untuk mendeteksi residu antibiotik pada daging ayam dan telur dari pasar tradisional di Kota Yogyakarta. Sejumlah dua puluh empat sampel daging ayam dan 24 sampel telur diambil dari 8 pasar tradisional di kota Yogyakarta. Sampel diperiksa di Balai Besar Veteriner Wates, Yogyakarta, Indonesia menggunakan metode *bioassay* untuk mendeteksi residu penisilin, aminoglikosida, makrolida, dan tetrasiklin. Metode ini menggunakan beberapa bakteri, seperti *Bacillus stearothermophillus*, *B. cereus*, *B. subtilis*, dan *Kocuria rizophila*.

Persentase hasil menunjukkan bahwa sampel 8,3% (2/24) dari ayam yang diuji positif mengandung residu antibiotik oxytetracycline. Sementara itu, sebanyak 75% (18/24) sampel telur positif mengandung residu antibiotik penisilin, residu positif aminoglikosida sebesar 12,5% (3/24) dan residu positif oxitetrasiklin juga sebesar 12,5% (3/24) .

Kata kunci: residu antibiotik, daging ayam, telur, *bioassay*

Abstract

Studies on antibiotic residues content in food of animal origin are currently needed to support veterinary public health programs. The present study was described bioassay method for the detection of antibiotic residues in chicken meat and eggs from traditional market at Yogyakarta City. A number of twenty-four chicken meat samples and 24 egg samples were taken from 8 traditional markets in Yogyakarta city. Samples were examined at Centre for Veterinary Wates, Yogyakarta, Indonesia using Bioassay methodfor screening detection of penicillin, aminoglycoside, macrolide and tetracycline residues. This bioassay method using some bacteria, such as *Bacillus stearothermophillus*, *B. cereus*, *B. subtilis*, and *Kocuria rizophila*A percentage of the results showed that 8.33% (2/24) samples of chickens tested positively contained the oxytetracycline antibiotic residues. Meanwhile, as much as 75% (18/24) samples of positive eggs contain penicillin antibiotic residues, positive residues of aminoglycoside amounted to 12.5% (3/24) and the positive residues of oxytetracycline also amounted to 12.5% (3/24).

Keywords: antibiotic residues, chicken meat, egg, bioassay

Introduction

Chicken meat and eggs are food commodities of animal origin-that are easily and commonly consumed by the people of Indonesia. The increasingly widespread use of antibiotics in poultry farms increases the potential for antibiotic residues in chicken meat and eggs, and can trigger antibiotic resistance or antimicrobial resistance (AMR). The presence of antibiotics in foods of animal origin needs to be monitored thoroughly since breeding, because some antimicrobials have potential health risks as shown in Table 1.

Table 1. Main classes of antimicrobials and potential risks (J. European Comission, 2010)

|  |  |
| --- | --- |
| Class | Health risks |
| Sulfamides | Allergies (with skin rashes), Sweet’s syndrome, DRESS syndrome, leukopenia |
| Quinolone | Immediate hypersensitivity reactions (urticaria, angioedema, anaphylaxis), exanthema, Sweet’s syndrome |
| Beta-lactamines | Immediate reactions: urticaria, angioedema, rhinitis, bronchospasm and anaphylaxis, haemolytic anaemia, neutropaenia, eosinophilia.  Skin rashes, Stevens-Johnson syndrome, Lyell’s syndrome |
| Tetracyclines | Drug hypersensitivity syndrome, drug-induced lupus erythematosus such as a rash, anaphylaxis, DRESS syndrome, Sweet’s syndrome |
| Aminoglycoside | Allergic contact dermatitis |
| Phenicols | Rare bone marrow suppression: aplastic anaemia |
| Macrolides | Rare |
| Lincosamides | Neuromuscular blockade with post-anaesthetic paralysis, cardiac depression after too rapid IV injection, allergies and moderate hepatic  degeneration |

DRESS syndrome: drug reaction (or rash) with eosinophilia and systemic symptoms syndrome

In past years, studies have been published that antibiotic resistant bacteria were found in food of animal origin (Ahlem, *et al*., 2007, Levertein-van Hall, *et al*., 2011, Geser, *et al*, 2012, Ambrozic-Avgustin, *et al*., 2012). The impact of antibiotic residues on food of animal origin can increase the potential threat to toxicological, microbiological, and immunological aspects in the human body. Among others, it can be toxic to the liver, kidneys and central system of haemopoietic, it also can disrupt the balance of microflora in the digestive tract and can trigger allergies (Riviere and Papich, 2009). Food from animals that contain antibiotic residues is still safe for consumption as long as it is below the standard of maximum limit residue of permissible (MRL). In Indonesia it has been regulated in SNI No. 01-6366:2000 (BSN, 2000).

Table 2. Maximum residue limit in chicken meat and eggs [7].

|  |  |
| --- | --- |
| Antibiotic | Maximum limit residue (mg/kg) |
| Penicillin | 0.1 |
| Oxytetracycline | 0.05 |
| Streptomycin | 0.1 |
| Erythromycin | 0.1 |

Meanwhile, the use of antibiotics as feed additives In Indonesia, are still widely common. Bahri *et al*. (2006) reported that the use of tetracycline and sulphonamide antibiotics as feed additives in chicken by 74.43% (5 of 7) feed factories in Bogor, Cianjur, Tangerang, Bekasi and Sukabumi Regencies (Bahri *et al*. ,2006). Meanwhile, Oramahi *et al.* (2004) has also reported that chicken liver in the city of Yogyakarta was contained antibiotics residue respectively 29.23% for penicillin, 36.92% for macrolide, 1.54% for macrolide and 26.15% for tetracycline. The results of other research studies on the assessment of the residues of several antibiotic groups in chicken eggs in several provinces in Indonesia showed that chicken eggs in Indonesia are still relatively safe for consumption because no residual content was detected in all egg samples tested (Nurhidayah, *et al*., 2015).

Therefore, since 2009 the Indonesian Government as prohibited the use of antibiotics including tetracycline as a feed additive through the Law Number 18 of 2009 regarding Livestock and Animal Health (Ministry of Agriculture Republic Indonesia, 2009).

Antibiotic residue testing can be done by rapid testing (Wehr and Frank, 2004). Screening tests with bioassays (Eennennaam, *et al*.,1993, Pikkemaat, *et al*., 2009, BSN, 2008). ELISA test (Wang, *et al*., 2009) and by using High Performance Liquid Chromatography (HPLC) (Wehr and Frank, 2004)]. Screening tests with bioassays are qualitative tests, which can be used easily, for large sample sizes, are not too expensive and the results of false negative tests are very small. ELISA and HPLC are common to analyse antibiotic residue quantitatively and specific to certain antibiotic (Zulfianti, 2005).

To ensure the food safety of food animal origin, this study aims to reveal the level of antibiotic residues of chicken meat and eggs circulating in the Yogyakarta region qualitatively by using bioassay method.

# materials and methods

## **Sample Collection**

Sampling was carried out from 8 traditional markets at Yogyakarta city by judgment method sampling. The total chicken meat samples taken were 24, obtained from breast and thigh parts and total eggs samples taken were 24. Sampling was carried out aseptically. Furthermore, the samples were taken to the Centre of Veterinary Wates, Yogyakarta, with a coolbox for testing the presence of antibiotic residues by bioassay method using *Bacillus stearothermophyllus*, *Bacillus cereus, Bacillus subtilis,* and *Kocuria rizophila.* During sampling, questioners were also distributed to the sellers in the markets.

**Screening testing using Bioassay method**

Bioassay is carried out using microorganisms to detect antibiotic compounds that are still active (BSN, 2008). The principle of this test is that the inhibition of bacterial growth by antibiotics contained in food of animal origin shows a positive effect on antibiotic residues (BSN, 2008). Bioassay method on chicken meat and eggs to test the content of antibioitic residues in this study was carried out by weighing samples of chicken meat and eggs each weighed as much as 10 g then added buffer number 2 as much as 20 ml of phosphate solution. Then homogenized using a homogenizer, then centrifuged 3000 rpm for 10 minutes. The supernatant is then taken and ready to be used as a test sample solution (BSN, 2008).

## **Preparation**— Antibiotic residues testing in chicken meat and eggs using bioassay screening test methods refer to SNI No. 7424: 2008, initiated with the making of agar media, preparation of culture media, preparation of buffer solutions, and preparation of standard solutions. The antibiotics used for this test were penicillin, aminoglycoside, tetracycline and macrolide groups.

The media making for the antibiotic groups were used peptone, yeast extract, bacto agar, aquadest, and KH2PO4, each following the protocol in SNI. Spesific bacteria were used for media culture preparation; for penicillin test used *Bacillus stearothermophillus* ATCC 7953, aminoglycoside test used *B. subtilis* ATCC 6633, tetracycline test used *B. cereus* ATCC 11778, and macrolide test used *Kocuria rizophila* ATCC 9341 [13]. Buffer phosphate solution preparation also refer to SNI No. 7424: 2008 (BSN, 2008).

**Samples examination**—At first, media cultured were prepared for each antibiotic’s examination. Next, the petri dish was added by 3 paper discs for each which one paper disc has already soaked into sample extract, one paper disc was dropped with standard solution as positive control, and one paper dish was dropped with buffer phosphate solution as negative control. All paper discs were laid on precisely at the superficial of the agar media. The petri dishes then were incubated in the incubator with the specific thermal for each antibiotic. Tetracycline groups need incubation thermal at 30°C ± 1°C, penicillin groups at 55°C ± 1°C, macrolide and aminoglycoside groups at 36°C ± 1°C along 16 to 18 hours. Each examination was repeated three times (triple examination). The results of the *assay* method for testing antibiotic residue were carried out by observing the inhibition zones that formed after the incubation period was completed and then measured in diameter using a caliper. The inhibition zone is the working effect of the presence of antibiotics which inhibits the growth of bacteria around the paper disk. The diameter of the inhibition zone formed shows the concentration of antibiotic residues (Pikkemaat, *et al*., 2009).

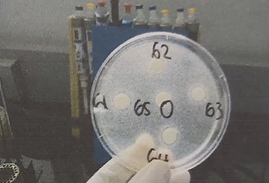
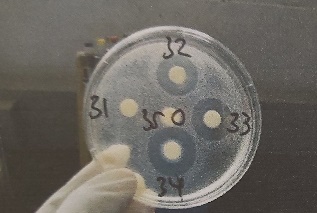
Positive results if the inhibition zone formed is 14 mm ± 1 by using a 10 mm diameter paper disc or 12 mm ± 1 in diameter by using an 8 mm diameter paper disc depending on the disc paper used.

# **results**

This study used 10 mm diameter paper discs, so it would be positive if the inhibition zone formed were ≥ 14 mm ± 1.

The results of the oxytetracycline residue by using bioassay test from chicken meat samples are shown in Fig. 1.

**a**



**b**



**c**

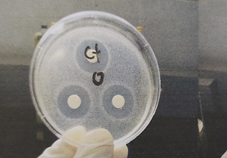
**d**

Fig. 1. Result of oxytetracycline residues by bioassay test in chicken meat samples.

(a).Positive result as inhibition zone formed were ≥ 14 mm ± 1. (b). Negative result as no inhibition zone were formed. (c). Positive control with oxytetracycline. (d). Negative control versus positive control of macrolide

The results of the antibiotic residue bioassay test from egg samples are shown in Fig. 2.

**a**



**b**



**d**

**c**

Fig. 2. Result of Antibiotic residues by bioassay test in egg samples.

(a).Positive result of pennicillin residue (b). Positive result of oxytetracycline residue. (c). Positive result of aminoglycoside residue (kanamycin). (d) Negative control versus positive control of oxytetracycline

The total results of tests on antibacterial residues in chicken meat and eggs for sale in traditional markets in the Yogyakarta region are shown in Table 3.

Table 3. Antibiotic residues examination result

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Number of positives from total sample (%) | | | | | | | |
| Chicken meat samples | | | | Egg samples | | | |
| AMG\* | MCR | PEN | OTC | AMG | MCR | PEN | OTC |
| - | - | - | 2/24  (8.3) | 3/24  (12.5) | - | 18/24  (75) | 3/24  (12.5) |

Information:

\*AMG: aminoglycoside

MCR: macrolide

PEN: penicillin

OTC: oxytetracycline

There were 2 samples of chicken meat containing oxytetracycline antibiotics and 3 samples of eggs positive for antibiotics in the group of aminoglycosides, 18 samples positive for penicillin, and 3 samples of eggs that were positive for antibiotic oxytetracycline.

# discussion

The results obtained showed that the antibiotic residues of penicillin, oxytetracycline and kanamycin from the aminoglycoside group were found in chicken and eggs sold in traditional markets in the Yogyakarta region.

Qualitative test results using bioassay method on chicken meat samples showed that 8.33% (2/24) samples contained oxytetracycline. Meanwhile, the test results of antibiotic residues in chicken eggs obtained 75% (18/24) samples of chicken eggs containing penicillin, oxytetracycline as much as 12.5% (3/24) and contained aminoglycoside groups as much as 12.5% (3/24) (Table 3). Meanwhile, the results of the questionnaire distributed to chicken and egg traders could not be determined with certainty because the traders only received from the company or broiler chicken breeders and no one was raising themselves.

The results above indicates that the use of antibiotics is still quite common in poultry farms in Yogyakarta area.

Oxytetracycline (OTC) is a broad-spectrum antibiotic in the tetracycline class which is widely used for the prevention and control of diseases in poultry industry (Zulfianti, 2005).  It can be used as a respiratory treatment and if the dose is low it can be used as a growth booster (Slana and Dolenc, 2013). Although the tetracycline group is only allowed as animal medicine and is not included in the feed additives that are permitted in Indonesia, this class is often used as feed additives. It is commonly used because of its many beneficial aspects, including its availability, relatively cheaper price, more easily use by oral administration through drinking water or feed, can increase the growth of broilers, and also can increase the efficiency of feed use in broilers (Slana and Dolenc, 2013, , Bachiri *et al*., 2017). The presence of OTC in chicken meat is likely caused by it characteristic, such as bacteriostatic; therefore it is difficult to be metabolized and partly excreted in the form of parent compounds due to its high solubility in water (Bachiri *et al*., 2017)

The presence of high penicillin antibiotic residues in eggs probably comes from the use of antibiotics continuously and for a long time through drinking water or feed in low concentrations. Penicillin can interfere with the synthesis of bacterial cell walls, consequently the bacterial cell wall ruptures. However, the use of penicillin that is too extensive is resistance to anti-drug drugs. Some groups of bacteria have antibiotic resistant properties, including *Escherichia coli* which can produce the enzyme penicillinase so that it can damage penicillin. In addition, some strains of *Staphylococcus aureu*s and *S. pneumoniae* are also resistant to penicillin. Several strains of *Enterobacteriaceae* are intrinsically aminopenicillin-resistant, particularly among *E. coli* species (Lobanovska and Giulia, 2017). Bachiri *et al*. (2017) reported that African wildlife can act as a reservoir of the epidemic *E. coli* clone ST131 producing CTX-M-15 that indicated the presence of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae in wild boars and Barbary macaques in Béjaïa and Jijel, Algeria.

The results of this study are still qualitative and need to be confirmed quantitatively by other methods such as HPLC, so that it can be known whether the antibiotic residual content is still within the maximum standard residual limit or even exceeds so that it can have an impact on human health. The results of this study also support previous studies that chicken liver in the city of Yogyakarta was contained mainly penicillin and tetracycline [9]. Nevertheless, the results of our studies are of interest since food animal origin, particularly chicken meat and eggs were shown to be a potent reservoir of multidrug-resistant organisms. Thus, the high prevalence of antibiotic residues could be a source of resistance developments among many bacterial strains. To decrease the resistance rate of bacteria, monitoring of resistance, surveillance, prudent use, research projects, awareness, and educational programs are recommended by WHO (2017). The application of strict regulations on the use of antibiotics in the field of chicken farming and monitoring the presence of residues on livestock products before they are marketed is very necessary to prevent the adverse effects of these residues on livestock origin food products.

##### Acknowledgment

Researchers would like to thank Faculty of Veterinary Medicine, Universitas Gadjah Mada for providing research funding. The researcher would also like to thank the head of the Centre of Veterinary Wates who had given the facility to conduct this study.

*“All authors declare that there are no conflicts of interest”*

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