

Research

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

Dyah Ayu Widiasih<sup>1\*</sup>, Yatri Drastini<sup>1</sup>, Doddi Yudhabuntara<sup>1</sup>, F. Lintang R. Daru Maya<sup>2</sup>, Prisha Lini Sivalingham<sup>2</sup>, Heru Susetya<sup>1</sup>, Widagdo Sri Nugroho<sup>1</sup>, M. Th. Khridiana Putri<sup>1</sup>, Roza Azizah Primatika<sup>1</sup>, Bambang Sumiar<sup>1</sup>

<sup>1</sup>Department of Veterinary Public Health, Faculty of Veterinary Medicine,  
Gadjah Mada University, Indonesia

<sup>2</sup>Faculty of Veterinary Medicine,  
Gadjah Mada University, Indonesia

\*Corresponding author: dyahaw@ugm.ac.id

Submitted 6 November 2019, Accepted 22 December 2019

### 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 method for screening detection of penicillin, aminoglycoside, macrolide and tetracycline residues. This bioassay method using some bacteria, such as *Bacillus stearothermophilus*, *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

### 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 stearothermophilus*, *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 oksitetrasiklin juga sebesar 12,5% (3/24).

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

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) (Table 2). In Indonesia it has been regulated in SNI No. 01-6366:2000 (BSN, 2000).

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 (Eennenam 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.

Table 1 Main classes of antimicrobials and potential risks (J. European Commission, 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.
Tetracyclines	Skin rashes, Stevens-Johnson syndrome, Lyell's syndrome 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

Table 2 Maximum residue limit in chicken meat and eggs

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

## MATERIAL 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 antibiotic 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  $\text{KH}_2\text{PO}_4$ , each following the protocol in SNI. Specific bacteria were used for media culture preparation; for penicillin test used *Bacillus stearothermophyllus* 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  $30^\circ\text{C} \pm 1^\circ\text{C}$ , penicillin groups at  $55^\circ\text{C} \pm 1^\circ\text{C}$ , macrolide and aminoglycoside groups at  $36^\circ\text{C} \pm 1^\circ\text{C}$  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  $14 \text{ mm} \pm 1$  by using a 10 mm diameter paper disc or  $12 \text{ mm} \pm 1$  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  $\geq 14 \text{ mm} \pm 1$ . The results of the oxytetracycline residue by using bioassay test from chicken meat samples are shown in Figure 1. The results of the antibiotic residue bioassay test from egg samples are shown in Figure 2. 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.

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

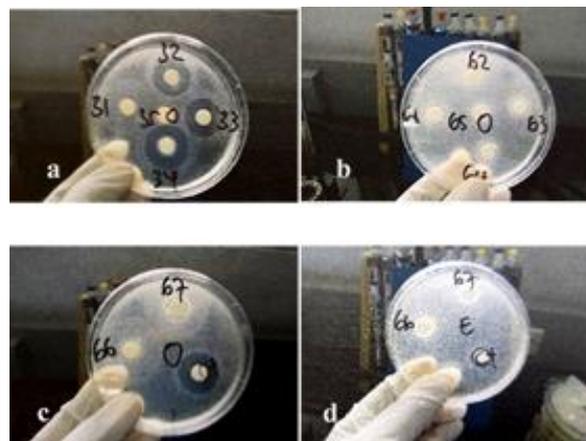


Figure 1 Result of oxytetracycline residues by bioassay test in chicken meat samples. (a) Positive result as inhibition zone formed were  $\geq 14 \text{ mm} \pm 1$ . (b) Negative result as no inhibition zone were formed. (c) Positive control with oxytetracycline. (d) Negative control versus positive control of macrolide

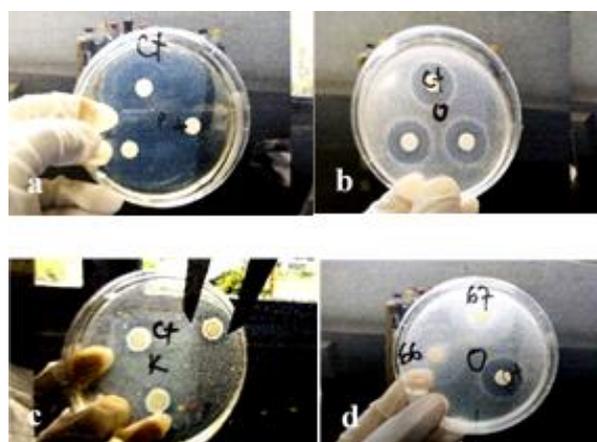


Figure 2 Result of Antibiotic residues by bioassay test in egg samples. (a) Positive result of pennicillin residue. (b) Positive result of oxytetracycline residue. (c) Positive result of aminoglycoside residue (kanamycin). (d) Negative control versus positive control of oxytetracycline

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

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 its 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 aureus* 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  $\beta$ -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.

## ACKNOWLEDGEMENTS

Researchers would like to thank Faculty of Veterinary Medicine, Gadjah Mada University 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”.

## REFERENCES

- Ahlem J, Vinué L, Ben Slama K, Sáenz Y, Klibi N, Hammami S, Boudabous A, Torres C. 2007. Characterization of CTX-M and SHV extended-spectrum  $\beta$ -lactamases and associated resistance genes in *Escherichia coli* strains of food samples in Tunisia. *J Antimicrobe Chemother.* 60: 1137-41.
- Ambrozic-Avgustin J, Zdovc I, Strumbelj I. 2012. *Escherichia coli* producing extended spectrum beta-lactamases: genotypes of isolates from diseased animals and food of animal origin compared with genotypes of human clinical isolates. *Medicinski Razgledi.* 51: 219-25.
- Bachiri T, Bakour S, Ladjouzi R, Thongpan L, Rolain JM, Touati A. 2017. High rates of CTX-M-15-producing *Escherichia coli* and *Klebsiella pneumoniae* in wild boars and *Barbary macaques* in Algeria. *J. Glob Antimicrob Resist.* 8: 35-40. doi: 10.1016/j.jgar.2016.10.005. Epub 2016 Dec 10.
- Badan Standarisasi Nasional (BSN). 2000. Batas Maksimum Cemaran Mikroba dan Batas Maksimum Residu Dalam Bahan Makanan Asal Hewan. SNI No. : 01-6366-2000. Jakarta.
- Badan Standarisasi Nasional (BSN). 2008. Metode Uji tapis (*Screening Test*) Residu Antibiotika pada Daging, Telur dan Susu secara Bioassay. SNI No. : 7424-2008. Jakarta.
- Bahri S, Sani Y, Indraningsih. 2006. Beberapa faktor yang mempengaruhi keamanan pangan asal ternak di Indonesia. *Wartazoa.* 16 (1): In Indonesian
- Eennennaam ALV, Cullor JS, Peran VL, Gardner A, Smith WL, Dellinoer J, Outerbocks WM. 1993. Evaluation of milk antibiotic residue screening test in cattle with naturally occurring clinical mastitis. *Dairy Sci.* 76: 3041-3053
- Geser N, Stephan R, Hächler H. 2012. Occurrence and characteristics of extended- spectrum  $\beta$ -lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. *BMC Vet Res.* 8. doi: 10.1186/1746-6148-8-21.
- European Commission (EC). 2010. Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *Off. J. Eur. Union, L 15*, 1–72. Available at: [http://ec.europa.eu/health/files/eudralex/vol5/reg\\_2010\\_37/reg\\_2010\\_37\\_en.pdf](http://ec.europa.eu/health/files/eudralex/vol5/reg_2010_37/reg_2010_37_en.pdf) (accessed on 13 August 2019).
- Levertein-van Hall MA, Dierickx CM, Cohen SJ, Voets GM, Munckhof van den MP, Essen-Zandbergen van A, Plateel T, Fluit AC, Sande-Bruinsma van de N, Scharinga J, Bonten MJM, Mevius DJ. 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clin Microbiol Infect.* 17: 873-80.
- Lobanovska M, Giulia P. 2017. Penicillin's discovery and antibiotic resistance: lessons for the future?. *Yale Journal of Biology and Medicine.* 90 (2017): 135-145
- Ministry of Agriculture Republic Indonesia. Directorate General of Livestock and Animal Health. 2009. The Law Number 18 of 2009 regarding Livestock and Animal Health.
- Nurhidayah, Nina TY, Maria FP, Novida A, Unang P, Sri W, Eli N, Rosana AN, Ambarwati, Dyah W, Emi R. 2015. Pengkajian residu beberapa golongan antibiotika pada telur di beberapa provinsi di Indonesia. *Buletin Pengujian Mutu Obat Hewan.* 23 tahun 2015.
- Oramahi R, Yudhabuntara D, Budiharta S. 2004. *Kajian Residu Antibiotik pada hati Ayam di Kota Yogyakarta.* Yogyakarta (ID): Program Studi Sain Veteriner, Sekolah Pasca Sarjana, UGM.
- Pikkemaat MG, Rapalini, MLBA, Dijk SOV, Elferink JWA. 2009. Comparison of three microbial screening methods for antibiotics using routine monitoring samples. *Analitica Chimica Acta.* 637 (2009): 298 – 314
- Riviere JE, Papich MG. 2009. *Veterinary Pharmacology and Therapeutics.* London (UK): Ninth Ed. Willey-Blackwell. pp 34-37
- Slana M, Dolenc MS. 2013. Environmental risk assessment of antimicrobials applied in veterinary medicine - a field study and laboratory approach. *Environ. Toxic. Pharm.* 35: 131-141
- Wang S, Xu B, Zhang Y, He JX. 2009. Development of enzyme-linked immunosorbent assay (ELISA) for the detection of neomycin residues in pig muscle, chicken muscle, egg, fish, milk and kidney. *Meat Sci.* 82: 53-58
- Wehr M, Frank JF. 2004. *Standard Methods for the Examination of Dairy Products.* Washington (US): American Public Health Association.
- World Health Organization (WHO). 2017. WHO Guidelines on Use of Medically Important Antimicrobials in Food- Producing Animals
- Zulfianti W. 2005. Penentuan kadar residu antibiotik dalam susu menggunakan bioassay. [Thesis]. Jakarta (ID): Program Sarjana, Universitas Islam Negeri Syarif Hidayatullah