Prevalence of *Salmonella* Spp. Bacteria Antibiotic Resistency Indigestion Tract in the Broiler Farms of Subang District

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ABSTRACT

This study is aimed to estimate the prevalence of resistant *Salmonella* spp., determine the spread of bacterial resistance and investigate the serotypes of bacteria in the chicken's digestion tract in the broiler farms in Subang District. As many as 74 farms were chosen, five poled caeca samples were taken from each farm and tested for isolation and identification of *Salmonella* spp. *Salmonella* isolates obtained were tested antimicrobial susceptibility against 8 antibiotics using the agar dilution method. The antibiotics were gentamycin, tetracycline, ciprofloxacin, nalidixic acid, ampicillin, chloramphenicol, trimethoprim, and sulfamethoxazole. The result showed that 8 out of 74 samples were positive for *Salmonella*. The prevalence of *Salmonella* spp. in the digestion tract was 10.8%; 95% confidence interval 3.7%-17.9%. Based on the serological test eight serotypes obtained were *Salmonella* enteritidis, *Salmonella oslo*, *Salmonella narashino*, *Salmonella nakuru*, and *Salmonella nordufer*. The result of antibiotic resistance test showed that from 8 *Salmonella* isolates obtained, 12.5% were found to be sensitive, 75% isolates were resistant to one or two antibiotics, and the remaining 12.5% isolates were resistant to more than two antibiotics; 95% confidence interval (0%-35.4%). The prevalence of resistant *Salmonella* spp. bacteria in chicken digestion tract in broiler farms in Subang District was high, so integrated control program to reduce antimicrobial resistance problem in broiler farm are greatly needed.

Keywords: Antibiotic resistance, Broiler farms, *Salmonella* spp. prevalence, Subang district

Introduction

Multidrug resistance (MDR) of foodborne pathogens such as *Salmonella* spp is a major concern for public health nowadays. imprudent use of antibiotics in broiler chicken farms increases the incidence of *Salmonella* spp. bacteria resistance. The spread of resistant bacteria can occur through direct or indirect contact, through food, water, and animal feces applied to agricultural land. *Salmonella* spp. resistant can spread to the environment through broiler feces and can transmit resistant genes to other bacteria, in line with Marshal and Levy (2011) which states that resistance can spread by horizontal transfer of genetic elements through conjugation, transduction and translation mechanism.

Resistant *Salmonella* spp. can enter the food chain through the animal origin food carrying resistant bacteria and contamination due to unhygienic animal origin food handling. Contaminated agricultural products due to the use of manure containing resistant *Salmonella* spp. can increase the risk of foodborne diseases caused by resistant bacteria (Hanning *et al.*, 2009).

The illness caused by *Salmonella* species was estimated 93,800,000 cases and resulting 155,000 human deaths globally in 2015 and estimated 85.6% of these cases are foodborne diseases (Eguale *et al.*, 2015). Subang district has about 7,959,370 broiler chickens population in 2016 (BPS 2018). Broiler chicken from farms in Subang district distributed to surrounding West Java and DKI Jakarta area. The incidence of foodborne diseases due to resistant bacteria is very important to control. Research needs to be done to estimate the prevalence of resistant *Salmonella* spp. to antibiotics used both in human medicine and broiler farm in Subang District.

Materials and Methods

This research was conducted from March to September 2018. The Isolation and identification of bacteria was carried out at the Subang Disease Investigation Centre Laboratory (BVet Subang) and serological (confirmation) testing of *Salmonella* spp. conducted at the Bogor Research Center For Veterinary Science (BBLitvet). Antibiotic resistance testing was carried out at the Bogor Quality Testing and Animal Product Certification Centre (BPMSPH).

Sampling was taken in partnership and independent broiler farms in Subang District using stratified random sampling method. The farms were selected by population criteria of 3,000 to 50,000 and at least 21 days old chicken. The results of broiler farm profiling obtained 126 eligible farms. The sample size was calculated by assuming a 95% of confidence level and 8% precision and the proportion (p) 0.5. The formula used was n = $4pq/L^2$, after obtaining n1 above 10%, the calculation of sample size was adjusted with the formula $= \frac{1}{\frac{1}{n1}+\frac{1}{N}}$, and the sample size was 74 samples.

Seventy-four samples of chicken cecum were tested for the identification of *Salmonella* spp. The method consisted of several steps, that is: (1) the selective enrichment stage used Hajna Tetrathionate Broth (HTTB), 1-2 ml sample Transferred to HTTB broth and incubated for 20 hours at 42°C; (2) one loopful HTTB mixture isolated to Deoxycholate Hydrogen Sulfide Lactose (DHL) Agar and incubated overnight at 37°C to select bacteria that can use lactose and sucrose and produce H2S; (3) no pink and red colonies from DHL agar isolated into mac Conkey agar (MCA) and incubated overnight at 37°C; (4) Bacterial colonies that grown on MCA was inoculated to triple sugar iron agar (TSI); (5) then the colonies tested by biochemical testing using lysine decarboxylase (LD), sulphite indol motility (SIM) and Simmons' citrate agar (Kishima, 2014). Positive isolates were continued to be Serological tested carried out in Bogor Research Center ForVeterinary Science.

Salmonella spp. Isolated from cecum samples were tested for antimicrobial susceptibility. The antimicrobial susceptibility was performed by disk dilution method following the Clinical and Laboratory Standards Institute protocol (CLSI, 2015). The antimicrobial susceptibility was tested against gentamicin (aminoglycoside class), tetracycline (tetracycline class), nalidixic acid, ciprofloxacin (fluoroquinolone class), ampicillin (penicillin class), chloramphenicol (chloramphenicol class), trimethoprim, and sulfamethoxazole (sulfonamide class). The method consisted of several steps, that is: (1) Inoculum and agar dilution preparation (2) Prepare a standardized inoculum for the agar dilution method by either growing *Salmonella* spp. to the turbidity of the 0.5 McFarland standard (3) Each standard of antibiotics solution

were taken as much as 1 mL and poured to a petri dish, then 9 mL Mueller Hilton media (MHA) added into a petri dish. (4) the 0.5 McFarland suspension diluted 1:10 in sterile broth or saline to obtain a concentration of 107 CFU/mL. (5) then the solution containing *Salmonella* spp. inoculated using a multi inoculator into the antibiotic-containing MHA medium and then incubated at 37°C for 18-24 hours. (6) The level of bacterial susceptibility is determined by comparing the smallest concentration of antibiotics that can inhibit bacterial growth with a minimum standard of inhibitory concentration (MIC) for each antibiotic.

Data on *Salmonella* spp. the resistance obtained from this study was analyzed descriptively by using Microsoft Excel. Prevalence data is estimated using the confidence level of 95% and calculated by the estimator formula of proportions as follows: $\hat{p} \pm z_{\alpha/2}\sqrt{\frac{p(1-p)}{n}}$.

Results and Discussions

Samples were taken from 74 farms, 71 partnership farms (44 types of partnerships) and 3 independent farms. This study found as many as 10.8%; (95% CI, 3.7-17.9%) isolating positive for *Salmonella* spp. from caecum samples. The serotype were *Salmonella* enteritidis as many as 4 isolates (50%), *Salmonella oslo, Salmonella narashino, Salmonella nakuru* and *Salmonella nordufer* each 1 isolate (12.5%) (Table 1).

The diversity of *Salmonella* serotypes that infect different host is strongly related to the ability of *Salmonella* serotypes to survive and thrive in different host. The Ability to survive from *Salmonella* spp. influenced by some interconnected factors, including differences in host environments such as pH, temperature, and binding sites and other factors (Foley *et al.*, 2013).

Salmonella oslo has been reported as a causative agent of food poisoning due to eating contaminated Persian cucumbers (Bottichio *et al.*, 2016). *Salmonella oslo* has been found in seafood made from squid and oysters originating from the southwest coast of India (Deekshit *et al.*, 2016) and also found in Harbor porpoises (Haase *et al.*, 2015) and pigs (EFSA, 2015). *Salmonella garba* has found in lizards (Bauwens *et al.*, 2006) and Shortnose crocodile (Reasoner, 1982).

Salmonella spp. has been known to have more than 2,500 serotypes spread in environment (WHO, 2018). This study found 5 *Salmonella* serotypes. Three serotypes could infect humans, the serotypes were *Salmonella* enteritidis, *Salmonella* oslo and *Salmonella* narashino, while *Salmonella* nakuru and *Salmonella* Nordfer have not been reported to cause infection in humans since 2006 (CDC, 2015). *Salmonella* isolates were tested to determine resistance to 8 types (6 class) of antibiotics using agar dilution method. The test results showed all isolates were sensitive to gentamicin, trimethoprim and chloramphenicol. The isolates were most resistant to ciprofloxacin,

tetracycline and nalidixic acid. It also experienced resistance to sulfamethoxazole and ampicillin (Figure 1).

Results of the antimicrobial susceptibility test showed 12.5% of *Salmonella* spp. Isolates were sensitive to all antibiotics tested. In general, resistance occurs in 2 classes of antibiotics. Multidrug resistance occurs in 12.5% *Salmonella* spp. Isolates (Figure 2). The resistance of *Salmonella* spp. known to be associated with the trend of subtherapeutic dose antibiotics in broiler farms (CDC, 2015). This study found resistance of *Salmonella* spp. occurs in farms that use 1 to 4 types of antibiotics in one cycle for disease prevention programme. According to Marshall and Levy (2011), antibiotic subtherapeutic uses are also clearly linked to the propagation of multidrug resistance (MDR), including resistance against drugs that were never used on the farm Imprudent use of antibiotics in animals husbandries can lead to antimicrobial resistance. The Increase of antimicrobial resistance leads to reduce the efficacy of antimicrobial, leading to increased morbidity, mortality, and health care expenditure. health care expenditure Increasing caused by additional treatment, longer hospitalizations, more complex diagnostic tests, higher professional service fees and other medical measures (CDC, 2015). According to Arisanti *et al.* (2018) in the period from 2000 to 2015, 61,119 foodborne deaseas were reported resulting 291 deaths (CFR 0.4%) in Indonesia, there were 1,176 outbreaks, most of it occurred in Central Java and West Java. The most causative agent was pathogenic bacteria (74.9%), 4.6% of outbreak caused by *Salmonella* bacteria.

The most risk factors were bad handling during food processing and improper storage. Resistant bacteria can be transmitted to humans through consumption of contaminated food from animals or through direct contact with infected animals and cross contamination. resistant *Salmonella* bacteria can lead to more seriously foodborne diseases. Resistant pathogens are able to cause serious disease and this is a major public health problem.

Resistance can arise spontaneously through the process of mutation, besides that resistant genes can be inherited or can be obtained from other bacteria through cellular genetic elements transfers such as plasmids. Horizontal gene transfer (HGT) can occur between bacteria so that the discovery of resistance events in *Salmonella* spp. in the environment in chicken house and digestive tracts on farms in the Subang District will increase the risk of resistance in other bacteria. This will be an important problem for human health given a large number of pathogenic bacteria that are spread in the environment and can freely contact bacteria that have experiencing resistance (Marshal and Levy 2011).

Table 1. *Salmonella* serotypes found in chicken digestion tract in broiler farms of Subang Regency

Serotipe	Sub-district	Resistence	Information
Salmonella enteritidis	Binong	-	Susecptible
Salmonella enteritidis	Compreng	TET, NAD	Resistant to 1-2 antibtics
Salmonella enteritidis	Cikaum	TET,CIP, NAD	Resistant to 1-2 antibtics
Salmonella enteritidis	Purwadadi	CIP, NAD, SUL	Resistant to 1-2 antibtics
Salmonella nakuru	Cipunagara	TET,CIP	Resistant to 1-2 antibtics
Salmonella narashino	Kasomalang	TET,CIP	Resistant to 1-2 antibtics
Salmonella nordufer	Pagaden	TET,CIP, NAD, SUL	Multi drugs resistent
Salmonella oslo	Kasomalang	CIP, NAD, AMP	Resistant to 1-2 antibtics

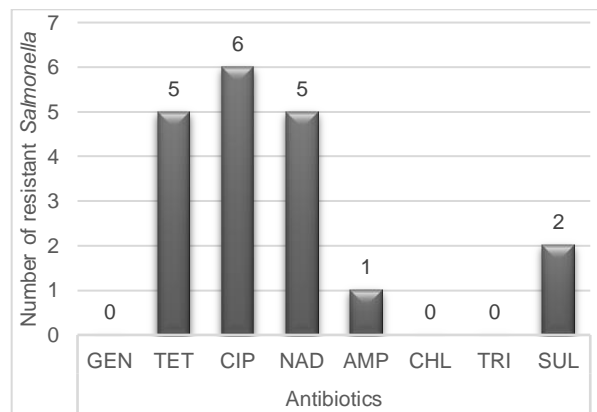Note: Tetracycline (TET), ciprofloxacin (CIP), nalidixic acid (NAD), ampicillin (AMP), sulfamethoxasol

Figure 1. Prevalence of antibiotics resistance of Salmonella spp. in digestive tract in broiler farms of Subang Regency. Gentamicin (GEN), tetracycline (TET), ciprofloxacin (CIP), acidic acid (NAD), ampicillin (AMP), chloramphenicol (CHO), trimethoprim (TMP), sulfamethoxasol (SUL).
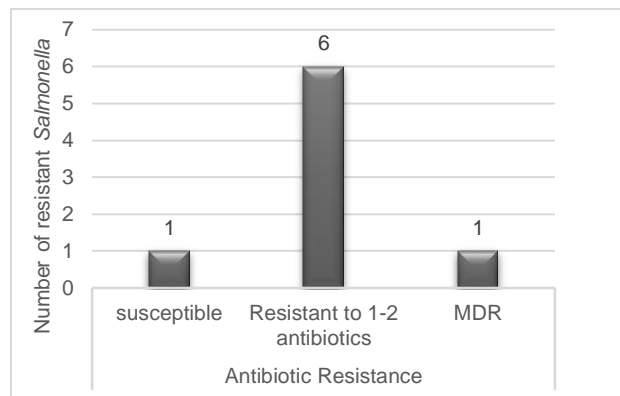
Figure 2. Pattern of Salmonella spp resistant in the gastrointestinal tract in broilers farm of Subang District.

This research collected samples from 26 sub-districts from 30 sub-districts in Subang District. Resistant *Salmonella* spp. was found in samples taken from 8 broiler farms in 7 sub-districts. It is shown that the resistant *Salmonella* spp. was spread in farms in the Subang district. It will affect the spread of bacterial resistance into the environment around farms and to other areas due to the traffic of broiler chickens for community consumption.

Conclusions

This study has found the rates of *Salmonella* spp. antibiotic resistance was quite high (9.46%) in the digestive tract of broiler chickens in Subang district. *Salmonella* serotypes found in the digestive tract are mostly bacteria that can cause disease in humans. *Salmonella* spp. most found to have experienced resistance to 2 classes of antibiotics to MDR. *Salmonella* spp. Antibiotic-resistant has found in 8 farms in 7 sub-districts that spread in Subang district.

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Acknowledgment

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