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Antibiogram Profile of *Salmonella Spp*. and Antimicrobial Residue of Chicken Egg in Yogyakarta: Implication to Public Health

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Abstract

Salmonellosis is one of the zoonoses that is transmitted through food or food-borne diseases, and causes a major public health problem worldwide. Currently, at the global level, the main sources of transmission for humans include contaminated poultry products, despite successful Salmonella controls applied in animal-feed production in industrialized countries. In recent years, shifts in Salmonella serotypes related to poultry and poultry production have been reported in various geographic areas, mainly related to the spread of certain well-adapted clones. In addition, antimicrobial resistance in non-typhoid Salmonella is considered as one of the major public health threats related to animal-food production, including the poultry production chain, which is a concern in the management of salmonellosis. The purpose of this study was to provide data on Salmonella originating from chicken eggs in retail outlets and layer farm in Yogyakarta. Hundred and sixty eggs were processed for Salmonella detection and isolate found were profile for antibiotic resistance. Most isolate were resistance to oxytetracycline (87.5%) and some were develop multi-drug resistance. Although the best poultry rearing system has not been a consensus, good management practice both in retail outlet and farms is highly required.

Keywords: egg; Salmonella; antibiotic resistance; antibiotic residue

Introduction

The poultry industry has been a remarkable national source of protein for Indonesian. Chicken meat and egg has become a staple food for all ages and levels of the community. It provides ease of processing and importantly affordable. The industry also endorses considerable economy in Indonesia (Ferlito & Respatiadi, 2018). The total layers population in 2020 is 81.2 million, centered in Central and East Java. Although this only constitutes 5% compared to 63% of broilers, in the total poultry population in Indonesia (Subdirectorate-of-Livestock-Statistics, 2020), the egg is constantly high in demand as a supreme component in the gastronomy industry (Islam et al., 2016)international, and public health agencies. A crosssectional study was conducted from July to December 2009 to detect the antibiotic residues in tissues and eggs of laying hens at Chittagong of Bangladesh. Materials and Methods: Microbial inhibition test (MIT.

In the shade of its great benefit lying potential threat to food safety related to animal welfare and care management. As food globalization and demand for a type of cuisine rapidly progressing globally, the public appears critically scrutinized and questioned the safety of the food. This urgency has contributed to massive research in food microbiology (Buncic, 2006).

Salmonellosis is a major foodborne disease threat worldwide sourced from poultry products. It is caused by non-typhoidal *Salmonella enterica* and normally the gastroenteritis signs improve after the incubation period between 4 -72 H. Although the bacteria are typically self-limiting, low mortality rates and life-threatening infections have occurred in risk populations (infants, young children, elderly, pregnant women, and immunocompromised people). The rise of resistance *Salmonella* to antibiotic drugs have posing risk to ineffective treatment (Antunes et al., 2016; Chen et al., 2013)invasive infections, such as bacteremia, osteomyelitis, and meningitis, may occur and require antimicrobial therapy. Continuous genetic and genomic evolution in Salmonella leading to increased virulence and resistance to multiple drugs are of significant public health concern. Two major changes in the epidemiology of nontyphoid salmonellosis in Europe and in the USA occurred in the second half of the 20th century: the emergence of foodborne human infections caused by Salmonella enterica serotype Enteriditis and by multidrug-resistant strains of Salmonella enterica serotype Typhimurium. In the 21st century, a worsening situation is the increasing resistance to fluoroquinolones and third-generation cephalosporins in nontyphoid Salmonella. Clinical isolates showing carbapenem resistance also have been identified. Although antimicrobial therapy is usually not indicated for uncomplicated Salmonella gastroenteritis, recent studies indicated that a short-course ceftriaxone therapy (3-5 days. Also, the belief in consuming raw eggs to boost vitality (Chusniati et al., 2009) increased attention to Salmonella infection.

Earlier animal welfare concepts were defined as the absence of disease, hunger, and thirst. However, the experience of positive states was later included to the extent the understanding of welfare science (Hartcher & Jones, 2017)animal welfare has been defined by the absence of negative states such as disease, hunger and thirst. However, a shift in animal welfare science has led to the understanding that good animal welfare cannot be achieved without the experience of positive states. Unequivocally, the housing environment has significant impacts on animal welfare. This review summarises how cage and cage-free housing systems impact some of the key welfare issues for layer hens: musculoskeletal health, disease, severe feather pecking, and behavioural expression. Welfare in cage-free systems is currently highly variable, and needs to be addressed by management practices, genetic selection, further research, and appropriate design and maintenance of the housing environment. Conventional cages lack adequate space for movement, and do not include features to allow behavioural expression. Hens therefore experience extreme behavioural restriction, musculoskeletal weakness and an inability to experience positive affective states. Furnished cages retain the benefits of conventional cages in terms of production efficiency and hygiene, and offer some benefits of cage-free systems in terms of an increased behavioural repertoire, but do not allow full behavioural expression. In Australia, while the retail market share of free-range eggs has been increasing in recent years, the majority of hens (approximately 70%. A welfare study of laying hens, emphasized positive experience aspects of cage-free chickens to express their natural behavior which absent in caged housing (Hartcher & Jones, 2017)animal welfare has been defined by the absence of negative states such as disease, hunger and thirst. However, a shift in animal welfare science has led to the understanding that good animal welfare cannot be achieved without the experience of positive states. Unequivocally, the housing environment has significant impacts on animal welfare. This review summarises how cage and cage-free housing systems impact some of the key welfare issues for layer hens: musculoskeletal health, disease, severe feather pecking, and behavioural expression. Welfare in cage-free systems is currently highly variable, and needs to be addressed by management practices, genetic selection, further research, and appropriate design and maintenance of the housing environment. Conventional cages lack adequate space for movement, and do not include features to allow behavioural expression. Hens therefore experience extreme behavioural restriction, musculoskeletal weakness and an inability to experience positive affective states. Furnished cages retain the benefits of conventional cages in terms of production efficiency and hygiene, and offer some benefits of cage-free systems in terms of an increased behavioural repertoire, but do not allow full behavioural expression. In Australia, while the retail market share of free-range eggs has been increasing in recent years, the majority of hens (approximately 70%.There was evidence of stress related to poor animal welfare practice in the increased case of Salmonellosis in poultry. A study in caged broiler confirming poor practice has induced animal stress led to higher susceptibility to microbial infections (Iannetti et al., 2020)posing microbial risks to consumers. Animal welfare levels, objectively measured with the application of the Welfare Quality® protocol, were assessed in thirteen broiler flocks, including organic ones, to evaluate the presence of statistically significant differences in relation to Campylobacter and Salmonella faecal shedding and consequent microbiological contamination of broiler carcases at slaughterhouse. Each flock underwent animal welfare evaluation the day before slaughtering, followed by Campylobacter and Salmonella detection in faeces (caecal content. There was a concern of season may affecting the Salmonella prevalence in free-range farm however a current study concluded no-significant effect (Gole et al., 2017). However, another study proved cold stress in the bird early life may impair the immune system long-term (Borsoi et al., 2015).

The utmost housing concerning Salmonellosis prevalence remains a controversy, and the incidence has resulted from a complex production system which required further investigation to underpin the definite causes (Whiley & Ross, 2015). In Indonesia, the commercial layer is reared in caged type housing. There is a very little knowledge of Salmonella contamination in egg in Indonesia in regard to welfare practice. The proposed aim of this research is to detect Salmonella contamination and antibiotic residue in chicken egg in Yogyakarta. Yogyakarta is located in Java island where layer farms are centered.

Materials and Methods

Ethical approval

Ethical approval was not necessary for the study. The samples were bought from the retail market and layers farm.

Study period and location

The study was conducted during August to November 2021. The bacterial examination and molecular analysis were processed in the laboratory of Department of Veterinary Public Health and Department of Microbiology, Faculty of Veterinary Medicine Universitas Gadjah Mada, Indonesia.

Sample collection

Hundred and sixty eggs which comprise of 5 eggs each pool was collected from seven retail outlet and six layers farm with population of >1000 in Yogyakarta, Indonesia. The samples were transported to the laboratory and stored at 4°C within 24H before analysis.

Salmonella Detection from Eggshell

The bacteriological analysis was carried out as described previously by Pande et al, 2016, Long et al, 2017 Gole et al, 2014 and Jajere et al, 2019 with modification. In brief, prior processing the egg was equilibrate under room temperature for 30 minutes. Five eggs were swabbed using wetted cotton sterile in Buffered Peptone Water (BPW, Oxoid) and immersed into 10 ml of Buffered Peptone Water (BPW, Oxoid) at 37◦C for overnight (18-24 H) pre-enrichment. The same procedure also done with Rappaport-Vassiliadis (RVS, Oxoid). The following day, 1ml of overnight BPW was transferred into 10 ml of Rappaport-Vassiliadis (RVS, Oxoid) then incubated at 42°C 24-48H. A loopful of 10µL ose was then streaked onto Xylose lysine deoxycholate and Brilliant Green Agar (BGA) plate at 37°C for 24H. The overnight swab in RVS was continued in XLD and BGA. Presumptive spherical transparent red halo with black center on XLD and moist spherical red/pink colonies with reddening media in BGA colonies was confirmed in Triple Sugar Iron Agar (TSIA, Oxoid). If Slant/Butt Red/Yellow with bubbles and black precipitate was observed the isolate then subculture in Nutrient Agar (NA, Oxoid) for further biochemical test as per the standard methods (Gole et al., 2017; Jajere et al., 2019; Long et al., 2017; Pande et al., 2016) Salmonella Typhimurium is frequently involved in egg and egg product related foodborne illness and Salmonella Mbandaka has also been found to be a contaminant of the layer farm environment. The ability possessed by Salmonella Enteritidis to colonize reproductive organs and contaminate developing eggs has been well-described. However, there are few studies investigating this ability for Salmonella Typhimurium. The hypothesis of this study was that the Salmonella Typhimurium can colonize the gut for a prolonged period of time and that horizontal infection through feces is the main route of egg contamination. At 14 weeks of age hens were orally infected with either S. Typhimurium PT 9 or S. Typhimurium PT 9 and Salmonella Mbandaka. Salmonella shedding in feces and eggs was monitored for 15 weeks post-infection. Egg shell surface and internal contents of eggs laid by infected hens were cultured independently for detection of Salmonella spp. The mean Salmonella load in feces ranged from 1.54 to 63.35 and 0.31 to 98.38 most probable number/g (MPN/g.

Salmonella Detection from Internal Egg

Subsequently after the eggshell swabbed then dipped into three parts of 70% alcohol to one part iodine tincture solution for 1 min to decontaminate from bacteria on the outside shell and allows to air dry.

The internal content of five eggs was aseptically removed into sterile falcon tube and homogenized thoroughly. One part of internal egg contents was then mixed in nine parts of BPW and another one part in nine parts of RVS, Oxoid for overnight (18-24 H) pre-enrichment at 37◦C. Following incubation, 100µL of the sample from BPW was transferred into RVS, Oxoid broth then incubated at 42°C for 24-48 h. A loopful is streaked onto BGA and XLD plates. The overnight sample in RVS was also streaked onto XLD and BGA. Presumptive colony growth was continued as in eggshell sample processing.

Antibiogram Profiling

The identified isolates were tested for their susceptibility to 7 antimicrobial agents: penicillin-G (P) - 10 µg, Ciprofloxacin (CIP) - 30 µg, Enrofloxacin (E) – 5 µg, Chloramphenicol (C) – 30 µg, Ampicillin (AMP) – 10 µg, Streptomycin (S) - 10 µg and Oxytetracyclin (OT) – 30 µg using the disk diffusion method (Bauer et al., 1996). In brief, the isolate was grown in Brain Heart Infusion broth (BHI, Himedia) for overnight at 37°C. The inoculated broth then adjusted to 0,5 McFarland and spread evenly onto a Mueller Hinton Agar (MHA, Oxoid). Antibiotic disc was placed on the agar surface and incubated at 37°C for 24H. The diameter of inhibition growth zone was measured and interpreted according to CLSI standard as R (resistance), I (Intermediate) and S (sensitve) (CLSI, 2019).

Detection of Antibiotic Residues

Antibiotic residues detection was employing Premi®Test (R-Biopharm, Germany) and conducted according to the company procedure. Briefly, the internal egg removal will be done as described previously. One-hundreds (100) µL of the 5 eggs pool sample was transferred onto the agar in the ampoules. After sealing with a porous lid, the ampoules then incubated at 80°C for 10 minutes and subsequently at 64°± 0,5°C approximately for 2 h 45 min. Samples were read after negative control ampoule change color. Samples from each brand from each supermarket were tested in duplo.

Statistical Analysis

A descriptive cross-sectional study is employed to determine the prevalence of Salmonella contamination in eggshell and internal contents of egg. The analysis and interpretation will be express as prevalence of disease in 2x2 tables and the relative risk.

Results

Antibiotic Residue

All egg samples were confirmed not contain any antibiotic residue.

Detection of *Salmonella* spp

The pools of eggshell and internal content from samples obtained from farms and retail outlets were 7,82% from eggshell while 10,95% from content (Table 1.). The layer farms comprised of 7,82% and retail outlets of 10,95%.

Table 1. Detection of Salmonella spp from layer farms and retail outlets in Yogyakarta.

Source	Pool	Shell (%)	Content (%)
Layer farms	24	2 (3,12)	3 (4,70)
Retail outlets	40	3 (4,70)	4 (6,25)
Total	**64**	**5 (7,82)**	**7 (10,95)**

Antimicrobial susceptibility

The antibiogram profile of Salmonella isolates were tested against seven antibiotics frequently

Table 2. The inhibition zone measurement category according to CLSI standard.

Isolates	S/I*	Type	Antibiotics						
			P	OT	CIP	E	AMP	C	S
Retail Outlets									
MP	S	bulk	R	S	I	S	I	S	I
H	S	bulk	I	R	S	S	S	S	S
H	I	bulk	S	R	S	S	S	S	S
ID	I	package	S	R	R	R	S	S	R
Farms									
BD	S		I	R	S	S	S	S	S
RY	I		R	R	R	R	S	S	R
RR	I		R	R	R	S	S	S	R
J	I		S	R	R	S	S	S	S

*S=shell, I=internal

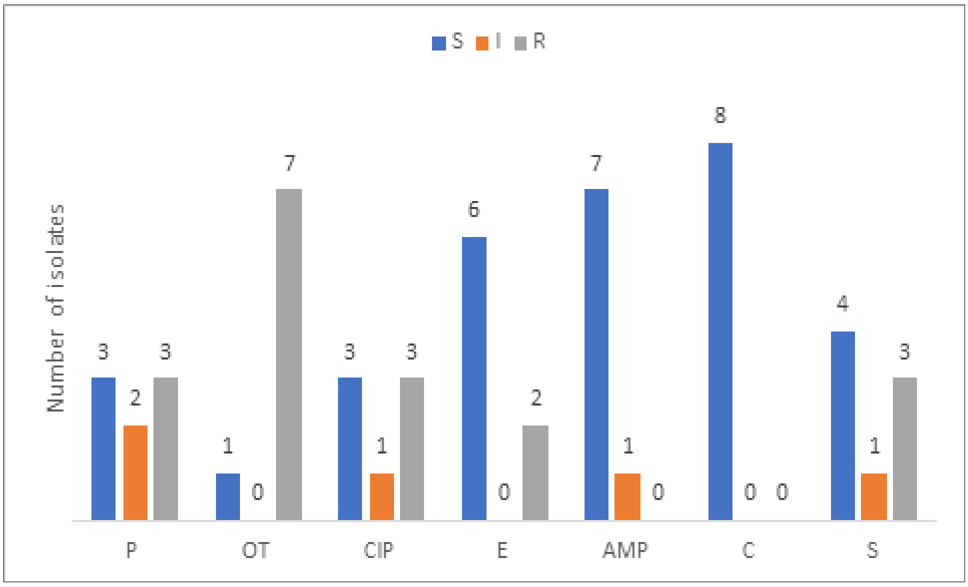

Figure 1. The number of isolates included in the resistance category for each antibiotic.

used in poultry farm (Table 2). The percentage of isolates (Figure 1) which sensitive to C, AMP, E, S, CIP, P and OT were 100%, 87.5%, 75%, 50%, 37.5%, 37.5%, and 12.5% respectively. In contrary, the majority of the isolates (87.5%) were resistance to OT while to antibiotic P, CIP, S and E were 37.5% and 25% respectively. The isolates which response intermediate to P, CIP, AMP, and S were 25%, 12.5%, 12.5% and 12.5% respectively.

Discussion

The pathogenic microbial contamination in food product has a serious impact in public health and food safety. It must consider thoroughly regarding human illness from mild symptoms to life-threatening stage. The prevalence of Salmonella isolate found on the eggshell is 7.82% whilst 10.94% was detected in the egg's internal content. Although the prevalence is low, the evidence shows underlying potential threat. In 2017, Salmonellosis has been responsible for 29% outbreaks and 34% of illnesses in US as a single etiologic agent (Center for Disease Control dan Prevention (CDC), 2019). This provides an insight of the importance of preventing contamination from this pathogenic agent. These microorganisms can be isolated both from eggshell and internal contents of eggs. The presence of Salmonella on the shell may be due to egg passes through cloaca which also an opening for digestive tract (Okorie-Kanu et al., 2016). However, the occurrence of the bacteria within the egg as a result from infected hen. It is demonstrated that both *S. enteritidis* and *typhymurium* are able to form colony within the reproductive tract of hen and cause egg production drop. There are variables influenced Salmonella contamination during egg production including flock size and age, stress, feed, vaccination, and cleaning practice. Research of rearing system has not yet been able to determine the impact to egg contamination (Whiley & Ross, 2015).

The susceptibility of the isolates found to seven antibiotics has suggested high resistance to Oxytetracycline (OT). A study in the semi-intensive poultry farm in Ethiopia showed 100% Salmonella resistance to oxytetracycline and ampicillin. This suggest the underdose treatment is still a common practice and long-term threat is not yet well understood. However, overdose antibiotic could create genomic pressure for microorganism to enable adaptation (Abda et al., 2021). Sub-lethal bactericidal antibiotic dosing for therapeutic promotes heterologous increase in resistance to other antibiotics. Common type of antibiotics employed in chicken farms for treatment are aminoglycoside, fluoroquinolone, tetracycline, sulphonamides – diaminopyrimidine, and lincosamides. Identified resistance antimicrobial drug should not be apply for treatment in the farms.

Conclusion

This study showed low prevalence of Salmonella contamination in chicken egg in Yogyakarta. However, Salmonella has potential as a single-etiology outbreak. The antibiogram profile showed concerns on emergence of AMR with multi-drug resistance pattern. A complex process of providing egg from farm to table showed a slightly higher prevalence of contamination in retail outlets. It is very important to create awareness for poultry breeders and retailers on the importance of good product management. A routine surveillance on the product for contamination is highly suggested in order to evaluate the management system.

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