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## Molecular detection of *Salmonella* in broiler chicken

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### Abstract

*Salmonella* is one of the infectious diseases that can found in broiler chicken. *Salmonella* is also a potential pathogen that can cause disease in both animal and human. This study was designed as reference for detection and identification of *Salmonella* bacteria from broiler chicken feces using culture methods and combined with PCR analysis. 10 fecal samples were isolated from close house type farm at Tabanan district, Bali, Indonesia. The samples are transported using Stuart media, enriched using Selenite broth and cultured in Xylose-lysine-deoxycholate (XLD) agar. After the *Salmonella* colonies grow, the DNA are extracted, proceed to PCR analysis and sent for sequencing. The result showed that 60% of the samples are *Salmonella enterica* bacteria that confirmed by culture media and molecular method.

**Keywords:** *Salmonella*, broiler chicken, feces, culture method, molecular method

### Introduction

Broiler chicken is a one of the livestock that is widely cultivated by breeders to meet the meat needs of consumers. Broiler chicken meat is the popular choice as food because it is cheap and easy to process. In order to provide the meat needs for the community and business purpose, many people start to open broiler chicken farm. The advantage of broiler chicken farming is the short production cycle, within 4-6 weeks broiler chickens can be harvested with a body weight of 1.5-1.56 kg (Yemima, 2014) [1]. However, there are many obstacles faced in producing healthy and quality chickens, one of the threats is infectious diseases and Salmonellosis are one of them.

Salmonellosis are caused by *Salmonella* bacteria that included in the *Enterobacteriaceae* family of bacteria, there are 2 species in the genus, *Salmonella enterica* and *Salmonella bongori*. More than 2500 serotypes have been identified which are differentiated based on their antigenic composition (Bopp *et al.*, 2003) [2]. Their size is about 2-5 µm long and 0.8-1.5 µm wide, straight rods, motile by peritrichous flagella, facultative anaerobic, and optimal growth temperature is 37 °C. D-Glucose and other carbohydrates are catabolized with acid and usually gas. They are oxidase negative, catalase positive, indole and Voges-Proskauer negative, methyl red and Simmons citrate positive. H<sub>2</sub>S is produced and urea is not hydrolyzed (Percival *et al.*, 2004) [3]. Apart from being economically detrimental, salmonellosis will also have a negative impact on public health. Although there are many other pathogens, the transmission of *Salmonella* through food will cause the emergence of a disease (Winarsih *et al.*, 2008) [4]. *Salmonella enterica* causes approximately 1.4 million cases of foodborne illness each year in the United States. Poultry and poultry products have been identified by several researchers as an important source of the majority of *Salmonella* infections in humans (Menconi *et al.*, 2013) [5]. Animals that suffer from salmonellosis can become persistent carriers. Besides being found in feces, *Salmonella* can also be isolated from soil, water and waste contaminated with fecal material from salmonellosis animals (Ray, 2001) [6].

From many reason that *Salmonella* is a potential pathogen that can cause disease in both animal and human, early detection is important steps to take and with using PCR analysis it

can increase the speed and efficiency of *Salmonella* detection (Singer *et al.*, 2006) <sup>[7]</sup>. Most studies support the detection of *Salmonella* using PCR analysis, as its specificity and sensitivity are superior to those of culturing (Thomas *et al.*, 2009) <sup>[8]</sup>. This study was designed for detection and identification of *Salmonella* bacteria from broiler chicken feces using culture methods and combined with PCR analysis.

## Materials and Methods

### Sample

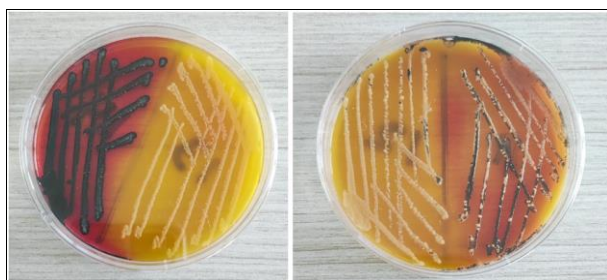
The samples are 10 random feces of finisher stage of broiler chicken in one close house type farm at Tabanan district, Bali, Indonesia. After the feces were taken, it transferred to Stuart media transport, and directly brought to the laboratory.

### Culture Method

After the sample was arrived at laboratory, the sample was transferred to Selenite media broth and incubated for 24 hours. After 24 hours, Selenite media will be cloudy, and it is ready to proceed to the Xylose-lysine-deoxycholate (XLD) agar for further identification. XLD agar is the most commonly used highly selective medium for the recovery of enteric pathogens from fecal specimens (Taylor, 1965) <sup>[9]</sup>. Some *Salmonella* produces black dots in the middle of the colony as a result of the production of H<sub>2</sub>S gas (Afriyani *et al.*, 2016) <sup>[10]</sup>. After the black colony of *Salmonella* grow, it will proceed to the molecular analysis.

### Molecular Method

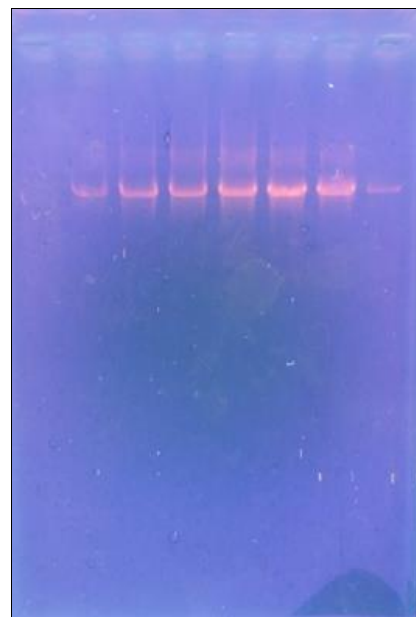
The colony were taken from the XLD agar and the DNA is isolated using GeneJET Genomic DNA Purification Kit and the PCR analysis for *Salmonella* bacteria are using (F:50 - ACAGTGCTCGTTTACGACCTGAAT-30, R:50 - AGACGGCTGGTACTGATT ATAAT-30) primer (Rozen & Skaletsky, 2000) <sup>[11]</sup>. After the DNA are obtain, the DNA will be sent for sequencing. The sequencing result will be proceeded to BLAST using MEGA 11 program to identify the *Salmonella* species.



**Fig 1:** *Salmonella* colony on Xylose Lysine Deoxycholate (XLD) with black color.

From 10 sample of fecal swab, 6 sample (60%) are producing black colony on XLD agar. The black colony identified as *Salmonella* bacteria according to Bell and Kyriakides (2002) <sup>[12]</sup> research that conclude the typical colonies of *Salmonella* on Xylose Lysine Deoxycholate (XLD) media are black in color, Zaraswati (2006) <sup>[13]</sup> also added statement that microbial colonies of *Salmonella* reduce thiosulphuric acid to sulfate so that the colonies appear black. But in this research, some of the XLD agar plates had yellow colonies instead of the expected red colonies with black centers. According to Leonard *et al.* (2015) <sup>[14]</sup> the yellow-pigmented colonies appear due to lactose fermentation by the microorganisms. *Escherichia coli* grow as yellow colonies on XLD while *Salmonella* is known as non-lactose fermenters and appear as

pink with black center colonies. There is some controversy however, with using XLD for *Salmonella* detection as there are *Salmonella* serovars which have horizontally inherited the lactose fermentation gene from *E. coli*. Several studies have also reported that *S. enterica* serovar *Typhimurium* and other *S. enterica* serovars which grow as yellow colonies on XLD agar (McDonough *et al.*, 2000) <sup>[15]</sup>. As this finding open many possibilities in the future for specific media agar that completely differentiate the *Salmonella* and *E. coli*. It is recommended that both microbiological culture methods and DNA molecular techniques are concurrently applied for the detection of *Salmonella* spp. even though culturing is more laborious and time consuming while molecular techniques are quick and more sensitive (Ahmed, 2014) <sup>[16]</sup>.



**Fig 2:** *Salmonella* PCR result

The PCR result showed a good band on all the positive samples, and the BLAST result are confirmed that the sample is *Salmonella enterica* with 92.39% similarity. *S. enterica* is responsible for infections in humans and animals, with serovars *Enteritidis* and *Typhimurium* being the most reported (Andino & Hanning, 2015) <sup>[17]</sup>. Avian salmonellosis can be broadly divided into two main types based on infection biology. The majority of broad-host range *S. enterica* serovars are capable of infecting the chicken, usually leading to a period of colonization of lower gastrointestinal tract (Beal *et al.*, 2004) <sup>[18]</sup>. *Salmonella* usually infects chickens via the fecal-oral route with spread from the intestinal tract primarily at the distal ileum and caeca of the bird (Barrow *et al.*, 2012) <sup>[19]</sup>. Although infection with these serovars can lead to systemic disease in chicks or immunocompromised animals, in healthy immunocompetent animals of a week of age or more, infection leads to little or no signs of disease (Wigley, 2014) <sup>[20]</sup>. In this research most of the clinical sign of the broiler chicken are not showing any sign of bacterial infection, probably it connected with the chicken immune system and their ages.

## Conclusion

From 10 fecal sample of broiler chicken, it is showed that 6 (60%) sample were positive with *Salmonella enterica* bacteria. Even there is some incompatibility identification using XLD media agar, but it was confirmed by molecular method.

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### Conflict

The authors declare no conflicts of interest regarding the publication of this paper.

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