

The Analysis of Bacteriology And Antibiotic Resistance to Salmonella Isolated in Fried Rolled Eggs at The Elementary School in Jatinegara District November-December 2017

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Abstract—Egg is one of the substances that is usually used to produce food. Street foods with egg as the main substance that are usually sold around an Elementary School located in Jatinegara district is *telurgulung* (fried rolled eggs). Salmonella is the bacteria that can be found in every food that contains egg. Salmonella can come from the hen as well as the surrounding environment. The purpose of this experiment is to identify Salmonella on street foods around Elementary schools located in Jatinegara district and to calculate the total of Salmonella found in *telurgulung* using Total Plate Count (TPC) method. This experiment aims to test the resistance of these Salmonella to the Amoxicillin, Chloramphenicol, and Ceftriaxone antibiotics by using Kirby Bauer method. According to the experiment that was done using 40 samples of fried rolled eggs street food, 2 samples found to be positively contained Salmonella, with the amount of colony $5,75 \times 10^3$ CFU/gr and $5,30 \times 10^3$ CFU/gr. According to *Keputusan Dirjen POM 2009*, the amount of Salmonella colony that found in this experiment can still be categorized to be in safe range, as it is still under the maximum limit of microbial contamination on food, which is not more than 1×10^4 CFU/gr. Antibiotics resistant test result obtained is according to the inhibition zone diameter formed, Amoxicillin showed the highest resistance (100%), whereas the sensitivity of Chloramphenicol and Ceftriaxone were found equal (50%). Chloramphenicol and Ceftriaxone were more effective in preventing the growth of the isolated Salmonella compared to Amoxicillin.

Keywords: Egg, Total Plate Count, Kirby Bauer, Salmonella

1. INTRODUCTION

Humans need foods to survive. According to the regulation of Republic of Indonesia Government no. 28 in 2004, the definition of food is everything that comes from biological sources and water both processed and unprocessed which is intended as food or drinks for humans' consumption. It includes food additives for raw materials and other materials used in the process of preparing, processing and or making food or beverages. Food that is useful for humans' survival is healthy food. Healthy food must be fresh and full of nutrition. If it is consumed in combination with other foods, it can sustain growth, improve and maintain vital processes, increase long life, reduce disease, and strengthen and maintain the body and its functions without containing toxic and bacterial contaminated material at the same time ¹

Foodborne disease is also referred to food poisoning. Salmonella is one of the most common foodborne disease bacteria that occurs in children. Disease caused by Salmonella bacteria called Salmonellosis with the main symptom is diarrhea. Foodborne disease is often found in snacks that is lack of hygiene ².

School-age children are in a group that is very vulnerable to food poisoning problems. Primary schools become places with a high incidence of food poisoning from year to year. According to BPOM RI elementary school contributed 18.75% of the total cases of food poisoning outbreaks in 2011. In Indonesia, the level of microbial pollution in PanganJajananAnakSekolah (PJAS) is generally still high. PJAS supervision data in 2008-2010 showed that 40-44% of PJAS did not meet the requirements of healthy foods, partly because they contained hazardous chemicals, food additives exceeded the safe limits and the presence of microbiological contamination ³

One of the most popular snacks for elementary school children is fried rolled eggs ⁴. Fried rolled eggs are one of the foods that contain high levels of protein. Bacteria will thrive on the foods with high protein compound compared to foods with the main form of carbohydrates. Salmonella is one of the bacteria which grows on animal foods ⁵.

In Indonesia the number of Salmonellosis case reached 1,213 cases in 2015 ⁶. In DKI Jakarta Province, from 10.5 million Jakarta residents, around 162 thousand of them suffer from Salmonellosis ⁶. One third of Salmonellosis cases in DKI Jakarta are in the area of East Jakarta. East Jakarta City has 10 sub-districts. Among these 10 sub-districts, the highest incidence of Salmonellosis is in Jatinegara sub-district ⁷.

The high cases of Salmonellosis in East Jakarta, especially Jatinegara are due to poor environmental hygiene and high poverty rates ⁶. Salmonellosis data tend to increase from year to year. This raises showed the possibility of antibiotic resistance for the treatment of Salmonella bacteria. There are several main antibiotics for treating Salmonellosis, including: Ciprofloxacin, Amoxicillin, Chloramphenicol, Cefixime, Azithromycin, and Ceftriaxone. Amoxicillin, Chloramphenicol, and Cefixime are safe for children to consume. In addition, the cheap and easy access to get Amoxicillin in the community is also a risk factor for resistance. Meanwhile, Ceftriaxone and Chloramphenicol are antibiotics that are the main choices in managing Salmonellosis. This is what underlies the researchers to test the resistance of these three antibiotics against Salmonella bacteria found from fried rolled eggs in this research.

2. MATERIALS AND METHOD

Research type

This research was an experimental descriptive study by examining the development of bacteria in vitro on culture media and counting the number of bacteria that grow in the media using TPC (Total Plate Count) technique. It was then identified by Gram staining. After the bacteria were identified, resistance testing was carried out against the antibiotics amoxicillin, chloramphenicol, and ceftriaxone using the Kirby Bauer method.

Research design

Testing Phase

a. Total Plate Count Method and Isolation of Bacteria

Samples that have been prepared (10 grams) were put into 90 ml NaCl (in an Erlenmeyer tube) then they were mixed well with vortex. After NaCl and sample were mixed, 1 cc of NaCl 90 ml was taken with micropipette, then it was put into the first test tube (dilution series). The process was repeated until the sixth test tube.

Next, 0.1 cc was taken from each test tube (10-1, 10-2, 10-3). It was then put into a petri dish that was filled with Salmonella Shigella Agar (SSA) media. The process was repeated until the third test tube and the results were put into each of the different SSA petri dishes. Next step was spreading or flattening the results using L stem.

After that, the bacteria was identified from isolated growth in SSA media. The bacteria were taken 0.1 cc from the first tube series and put into specific media. Then it was levelled with round one. The process was repeated in each medium. Then the bacteria were incubated for 18-24 hours at 37°C. Then the colony was calculated with the Colony counter-based on BPOM.

b. Gram staining

Gram staining can also be done from specific media (SSA) to identify the pathogenic bacteria contained. The steps to do Gram stain are:

A clean glass object was taken and fixed in Bunsen burner. 0.9% NaCl was dropped into the glass object. The aseptically colonized colonies were taken and spread in the glass object until they are thin and dried. This preparation is glued over a 2-3x bunsen. The smear was gently poured with crystal violet and let it be for 5 minutes. The smear was then rinsed with distilled water. The smear was gently poured with Gram's iodine and let it be for 5 minutes. The smear was then washed with water. 95% ethyl alcohol or acetone was used to decolorize it before it was washed with water. It was poured safranin to counter-stain and let it be for 45 seconds before it was washed with water and dried with filter paper. Finally it was dripped with 1 drop of

emersion oil and then it was viewed under a microscope with an enlargement of 10 x100.

c. Antibiotic Resistance Test

Colonies were taken from specific media by round. Then they were put into a test tube containing 0.9% NaCl then vortex to homogenize. Then the clarity of 0.9% NaCl contained in bacteria in the test tube was equated with McFarland 0.5 turbidity standard by adding 0.9% NaCl to the same clarity. After it was cleared, the colony was taken from the test tube containing 0.9% NaCl with a swab then it was applied to the MHA solid medium. After that, antibiotics (Amoxicillin, Chloramphenicol, and Ceftriaxone) in the form of paper discs were soaked in MHA media. Then, they were incubated for 18-24 hours at 37°C. Finally, the diameter of the inhibitory power formed around the paper disc (antibiotics) was measured using an electronic digital calliper.

d. Assessing Cultivation Results

The diameter of the inhibitory zone around the antibiotic disc was measured using an electronic digital calliper, then it was adjusted to the nature category of antibiotics according to the CLSI table which is an international standard in assessing the diameter of the inhibitory zone formed.

Population and Sample

The population of this study were all fried rolled eggs snack foods found in the elementary schools of Jatinegara sub-district in the period November - December 2017. Based from the survey there were only 40 merchant of fried rolled eggs snacks from 95 elementary schools located in the Jatinegara sub-district (total sampling).

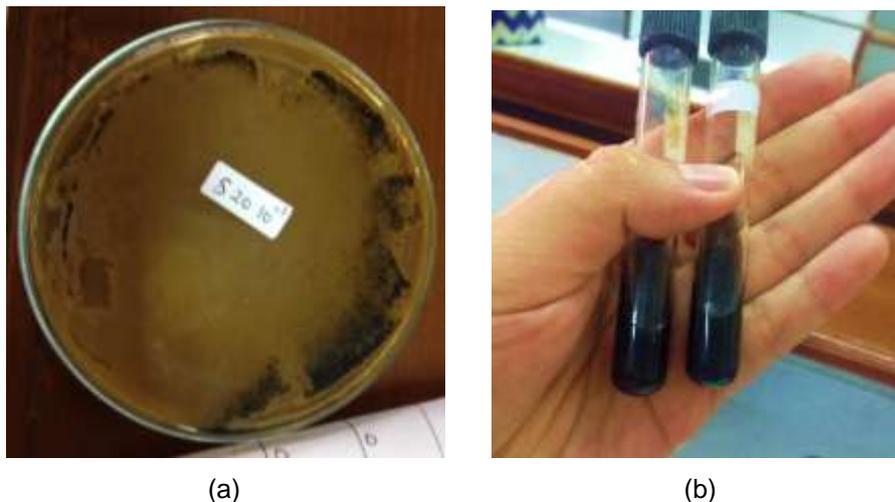
Sample Criteria

1. Fried rolled eggs snacks merchant in elementary schools in Jatinegara sub-district.
2. The sample taken is a cooked mixture of fried and rolled eggs.
3. Samples of fried rolled eggs snacks that are taken are those that traders will sell on the day of sampling.

3. RESULTS AND DISCUSSION

Results of the Inspection of Salmonella Contamination in Fried Rolled Eggs Snacks

Macroscopic test from the sample showed that from 40 samples, sample no. 20 and 28. Additionally, from the macroscopic test showed that *Salmonella* did not ferment lactose, but it produced hydrogen sulfide (H₂S) gas. The bacterial colonies would appear colorless with black centers and according to Triple Sugar Iron Agar Test (TSIA) on the picture 2.



Picture 1. Bacteria isolated growth in Salmonella Shigella Agar (a) and Triple Sugar Iron Agar Test (b)

From microscopic is test by using Gram stain to samples, and found the result as showed bacil, Gram negative (picture 2).



Picture 2. Gram stain from samples

Table 1 Average Number of Colonies for Each Sample of Fried Rolled Eggs Snack in Elementary Schools in Jatinegara Sub-District

Sample	Action of Column Amount (CFU/gram)	Information
20	$5,75 \times 10^3$	Not exceeding threshold
28	$5,30 \times 10^3$	Not exceeding threshold

It could be seen that the samples no 20 and 28 did not exceed the threshold determined by the Decree of the Director General of POM in 2009, with the maximum limit of the number of bacteria in food was 10^4 CFU / gram. Samples of 20 and 28 were the samples with the highest number of colonies, amounting to $5,75 \times 10^3$ and $5,30 \times 10^3$ while the rest of the other samples found no colonies of Salmonella bacteria.

Table 2. Results of Gram Staining Samples of Fried Rolled Eggs Snacks in Elementary Schools in Jatinegara Sub-District

Sample	Form	Arrangement	Color	character
No 20	Bacil	Single	Red	Gram Negative
No 28	Bacil	Single	Red	Gram Negative



Picture 3. Antibiotic Resistance Test

After Salmonella from the samples were identified, the antibiotic resistance showed a clear zone area around the paper disc containing antibiotic. It showed the inhibition zone for Chloramphenicol and Ceftriaxone.

Table 3. Salmonella AntibioticResistance Test Results

Antibiotic	Sample	Inhibition Zone Diameter (mm)	Note
Amoxicillin	20	11.5	Resistance
	28	7.5	Resistance
Chloramphenicol	20	20.5	Sensitive
	28	17	Intermediate
Ceftriaxone	20	24.5	Sensitive
	28	20	Intermediate

Discussion

Laboratory tests on fried rolled eggs snack food samples were carried out in two stages: examination of the plate count method and Gram staining. The examination of the plate count method aims to identify and count the number of Salmonella. The second examination is Gram staining done to ensure that the bacteria obtained in the SSA method are Salmonella based on the characteristics and bacterial properties obtained, namely a single stem and red with Gram negative properties. From the two samples, both showed the traits and properties that were in accordance with the Salmonella Genus.

Based on the examination of fried egg snacks, out of 40 fried rolled eggs food samples, two samples (5%) were positively contaminated by Salmonella, with the number of colonies found was 5.75×10^3 in samples 20 and 5.30×10^3 in samples 28. Based on the decision of the Director General of POM in 2009, the safe limit of bacterial contamination in prepared food is 1×10^4 CFU / g. In this research, the number of Salmonella obtained was quite low and still met the safety requirements ^{2,8}.

Salmonella bacteria contained in eggs can come from the hen itself such as Salmonella enteritidis and can also come from the outer environment of the egg that enter through a cracked eggshell. The principle of hygiene and sanitation of egg management can also affect egg contamination by Salmonella. Management of eggs as food must be in line with the principles of hygiene and good sanitation. Besides, the process of cooking eggs must also be at an optimal temperature. This is done to prevent contamination of food by Salmonella which may be found in eggs or from the environment ⁹.

Salmonella bacteria with low amounts in food indicate that the level of food safety and microbiological quality that is still quite good. This can be influenced by a good egg management process and optimal temperature so that Salmonella bacteria contained in it have been inactive. Based on the theory of hygiene principles and food management sanitation, a good food management process that meets health requirements can reduce the level of contamination ¹⁰

This is in accordance with the results of a previous study conducted by CDC in 2017 stating that maintaining cleanliness rather than selecting up to the egg management process can reduce the risk of egg contamination by Salmonella bacteria ⁹.

Based on the results of hygiene inspection and sanitation in the management of fried rolled eggs in 40 elementary schools in JatinegaraSubdistrict, it is known that most of the traders actually manage these snacks in accordance with hygiene and sanitation principles which are quite good. Although the snacks sellers might not have enough education about the importance of hygiene and sanitation principles in food management, they did good frying process and serving that can reduce the occurrence of the disease.

The results of this study indicated that Amoxicillin was resistant to Salmonella contained in fried rolled eggs snacks in elementary schools in Jatinegara District. This was indicated by the results of the average diameter of the inhibitory zone formed when antibiotic resistance testing is 11.5 mm and 7.5 mm. Both were classified as 100% resistant categories according to the 2014 CLSI table.

Chloramphenicol has a sensitivity value of 50% against Salmonella. This is caused by the diameter of the inhibitory zone found in one of the Chloramphenicol antibiotic test samples valued at 20.5 mm. These results were classified as sensitive categories according to the 2014 CLSI table. ¹¹

The second sample tested for antibiotics using Chloramphenicol showed the results of an average diameter of the inhibitory zone formed by 17 mm. These results indicated that Chloramphenicol could be classified as an intermediate category with an intermediate value of 50% according to the 2014 CLSI table. ¹¹

The results obtained are in accordance with the results of research conducted by Irma Suwasti, et al in 2008. The study examined the sensitivity of *Salmonella typhi* to antibiotics Chloramphenicol in Saiful Anwar Regional Hospital, Malang. The results found showed that *Salmonella typhi* was sensitive to Chloramphenicol antibiotics.

Ceftriaxone is a broad-spectrum antibiotic that is bactericidal (kills bacteria). Ceftriaxone bactericidal effects are produced due to inhibition of bacterial wall synthesis. Ceftriaxone has high stability against beta-lactamase, both penicillin and cephalosporins produced by Gram-negative and Gram-positive bacteria¹². However, with the use of Ceftriaxone antibiotics that are not in accordance with the proper rules, this can increase the risk of mutations in *Salmonella* bacteria so that it weakens the ability of Ceftriaxone drugs to kill the bacteria. As a result, the number of resistance to Ceftriaxone increases.

4. CONCLUSION

Based on the results of the research conducted on bacteriological testing and antibiotic resistance, amoxicillin, chloramphenicol, and ceftriaxone in *Salmonella* bacteria found in fried rolled eggs snacks in primary schools in JatinegaraSubdistrict, it can be concluded as follows:

- a. From the 40 samples studied, two samples of fried rolled eggs snacks in SD Jatinegara Sub-district were positively containing *Salmonella*, with the TPC method known the number of each colony was 5.75×10^3 and 5.30×10^3 .
- b. *Salmonella* bacteria found in fried rolled eggs food in elementary schools in Jatinegara sub-district were 100% resistant to Amoxicillin.
- c. *Salmonella* bacteria found in fried rolled eggs snacks in elementary schools in Jatinegara sub-district were 50% sensitive and 50% intermediate to Chloramphenicol.
- d. *Salmonella* bacteria found in fried rolled eggs snacks in elementary schools in Jatinegara sub-district were 50% sensitive and 50% intermediate to Ceftriaxone.

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REFERENCE

1. Podrabsky M, Quinn E. Improving Access to Healthy Foods in Washington State : A Policy Feasibility Study WA Policy Feasibility Study WA Policy Feasibility Study policies with reported potential for increasing • Compare and contrast perceptions of various. 2011;(July):1-8.
2. Nutrition C for FS and A. Consumers - Foodborne Illnesses: What You Need to Know. <https://www.fda.gov/Food/ResourcesForYou/Consumers/ucm103263.htm>. Accessed November 3, 2018.
3. *Direktorat Surveilan Dan Penyuluhan Keamanan Pangan, 2011.* <http://www.pom.go.id/ppid/2015/rpusat/spkp.pdf>. Accessed November 4, 2018.
4. Bonang KE&. *Mikrobiologi Kedokteran Untuk Laboratorium Dan Klinik.* gamedia, Jakarta; 2016.
5. Acdc. *PART IV ACUTE COMMUNICABLE DISEASES.* <http://publichealth.lacounty.gov/acd/procs/b73/B73Part4.pdf>. Accessed November 4, 2018.
6. profil kesehatan indonesia 2015. <http://www.depkes.go.id/resources/download/pusdatin/profil-kesehatan-indonesia/profil-kesehatan-Indonesia-2015.pdf>. Accessed November 4, 2018.
7. Ministry of Health of Indonesia. Roadmap to Healthy Indonesia 2010 (Indikator Indonesia Sehat 2010). 2000:1-40.
8. *BADAN PENGAWAS OBAT DAN MAKANAN REPUBLIK INDONESIA.* http://standarpangan.pom.go.id/dokumen/peraturan/2016/PerKa_BPOM_No_16_Tahun_2016_tentang_Kriteria_Mikrobiologi_dalam_Pangan_Olahan.pdf. Accessed November 4, 2018.
9. Salmonellosis. <https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/Salmonellosis.aspx>. Accessed November 3, 2018.
10. Undang-undang. PP NO 28 Tahun 2004. 2004.
11. Standart CLSI. CLSI, editor (2013), Clinical and Laboratory Standards Insitute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-third Informational Supplement M100-S23. CLSI, Wayne, PA,

USA.

<https://www.researchgate.net/file.PostFileLoader.html?id=581d9d8fcbd5c2f99c04d4b1&assetKey=AS%3A424985668919296%401478335887189>. Published 2015.

12. G. KB. *Basic and Clinical Pharmacology*. 10th ed. McGraw Hill. Boston; 2007.