Indonesian Journal of
Tropical and Infectious Disease
Vol. 5. No. 6 September–December 2015

Research Report

EVALUATION OF *Salmonella* sp CONTAMINATION AND ITS ANTIBIOTICS RESISTANCE PATTERNS ISOLATED FROM BROILER MEAT SOLD AT WET MARKET IN CENTER OF SURABAYA

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ABSTRACT

Antibiotic resistance now days become a main issue to the medical researches as found many positive result of antibiotic resistance test. One of the causes of antibiotic resistance is using antibiotic as a feed additive to animal. Bacteria that are resistant to antibiotics can be a danger to humans, in this case the resistant bacteria as a result of treatment errors animals, especially chickens that uses low-dose antibiotics as growth promoters. This study aimed to determine the contamination of Salmonella sp and its antibiotics resistance patterns of Salmonella sp isolated from broiler meat sold at wet market in the Center of Surabaya: (a) Pasar Kembang, (b) Pasar Kupang, (c) Pasar Dukuh Kupang, (d) Pasar Kedungsari, (e) Pasar Kedungdoro and (f) Pasar Keputran. The method that used in this study was bacteriological isolation and identification method. The method started with pre-enrichment using Buffered Pepton Water, selective enrichment using Tetrathionate Broth and Selenite Cysteine broth, selective media using Salmonella-Shigella Agar, Biochemical test using Triple Sugar Iron Agar, Simon Citrate, Methyl Red – Voges Proskauer, and Sulfide Indol Motility, and followed with susceptibility test according to Kirby-Bauer method using Mueller-Hinton Agar. The antibiotics that used in susceptibility test were: (a) Meropenem, (b) Ampicillin Sulbactam, (c) Amikacin, (d) Ofloxacin and (e) Nalidixic Acid. The results of this study were found 90% or 27 of 30 samples positive contaminated with Salmonella sp. The results of antibiotics resistance from 27 isolates 0% were resistant to Meropenem, 0% were resistant to Amikacin; 3.7% were resistant to Ampicillin-Sulbactam; 11.1% were resistant to Ofloxacin and 44.4% were resistant to Nalidixic Acid.

Key words: *Salmonella sp, wet market, broiler meat, antibiotic resistance, Center of Surabaya*

ABSTRAK

Resistansi antibiotik sekarang menjadi isu utama pada penelitian medis seiring ditemukannya banyak hasil positif pada uji resistansi antibiotik. Satu dari penyebab resistansi antibiotik adalah penggunaan antibiotik sebagai makanan aditif pada hewan. Bakteri yang resistan terhadap antibiotik dapat membahayakan manusia, pada kasus ini resistansi bacteria merupakan hasil dari kesalahan perlakuan pada hewan, terutama pada ayam yang menggunakan antibiotik dosis rendah sebagai pemicu pertumbuhan. Penelitian ini membahas mengenai cemaran dan resistensi terhadap antibiotika dari bakteri Salmonella sp yang diisolasi dari daging ayam broiler di Pasar Tradisional Surabaya Pusat (Pasar Kembang, Pasar Kupang, Pasar Dukuh Kupang, Pasar Kedungsari, Pasar Kedungdoro dan Pasar Keputran). Penelitian ini menggunakan metode isolasi dan identifikasi bakteri yang dilanjutkan dengan uji sensitivitas antibiotika menggunakan metode difusi dari Kirby Bauer. Antibiotika uji yang digunakan pada uji sensitivitas adalah: (a) Meropenem, (b) Ampicillin Sulbactam, (c) Amikacin, (d) Ofloxacin dan (e) Nalidixic Acid. Hasil dari penelitian ini adalah ditemukan

27 dari 30 sampel positif terkontaminasi bakteri Salmonella sp. Hasil uji sensitivitas terhadap antibiotika, 0% resisten terhadap antibiotik meropenem dan amikacin; 3,7% resisten terhadap antibiotika ampicillin sulbactam; 11,1% resisten terhadap ofloxacin dan 44,4% resisten terhadap nalidixic acid.

Kata kunci: *Salmonella sp, pasar basah, daging ayam broiler, resistensi antibiotika, Surabaya Pusat*

INTRODUCTION

The poultry product consumption especially broiler meat is predicted will climb up as increaseas the number of Indonesian population, lifestyle changes and the high awareness of the importance of protein consumed. On 2008, broiler meat consumption got up to 3,8 kg/capita/year. The total of broiler meat consumption reached at 84.07% from total consumption of the other livestock.[1] Broiler meat is a product that easy contaminates with pathogenic or non-pathogenic microorganism.[2] One of the microorganisms that often contaminate broiler meat is *Salmonella* sp, a bacteria caused Salmonellosis and recorded as the main cause of food borne disease.[3] There are 21.6 million cases of Salmonellosis in the world with 216.000 victim dies, and more than 90% happened in Asia.[4] Directorate General of Medical Services, Indonesian Department of Health in 2008 reported typhoid fever was on second rank of the top ten main diseases of inpatients in Indonesia's hospitals with 81.116 cases (proportion 3,15%), the first rank was occupied by diarrhea with the amount of 193.856 cases (proportion 7,52%).[5]

As the high level of demand for broiler meat, many farmers choose a shortcut way to increase the chicken's perform with giving feed additive, such as antibiotic to fast the growth of the chicken. Monitoring and surveillance in 2004 at Padang and Palembang reported that there were chicken, meat, and egg contained antibiotic residues. In Padang, from 98 specimens were found 3% contained tetracycline residues and 2% contained aminoglycoside residues. In Pekanbaru, from 22 specimens were found 4,8% contained penicillin residues.[6] 317 *Salmonella* sp isolated from Immanuel Hospital in Bandung were testedthe resistance of antibiotic and found that resistant to trimetoprim-sulfametisazol (7,89%), trimetoprim (6,95%), ciprofloxacin (4,11%), chloramphenicol (0,95%), and amoxicillin (0,62%).[7]

Seeing the potential incidence of salmonellosis and broiler meat as the media vulnerable to contamination by bacteria and the phenomenon of antibiotics as a feed additive for maintenance broilers, the researchers wanted to know the existence of contamination of *Salmonella* sp in broiler chicken meat sold in wet markets in the center of Surabaya and its antibiotic resistance against Salmonella sp.

MATERIAL AND METHODS

A total of 30 specimens (*musc. Pectoralis*) were collected randomly from 7 wet markets at Center of Surabaya between November–December, 2014. The list of wet market presented in Table 1.

Bacterial test including isolation, identification and susceptibility test were done at Gastroenteritis and Salmonellosis Laboratory, Institute of Tropical Disease, Airlangga University.

The bacteriological test started with pre-enrichment, 25 gram specimen put into an Erlenmeyer with 225 ml Buffered Peptone Water sterile (OXOID®) and incubates for 24 hours at 37°C.[8] The next day, inoculate 1ml isolate from pre-enrichment media to selective enrichment media using 10 ml Tetrathionate Broth (BD) and 10 ml Selenite Cystine Broth (BD), incubate for 24 hours at 37°C.[8]

The culture from each selective enrichment media were inoculated on selective media: Salmonella Shigella Agar (OXOID®) sterile with streaking using sterile loop on the surface of the plate, incubate all media for 24 hours at 37°C.[8] Biochemical tests were started with colony selection. Colonies that showed suspect of *Salmonella* sp were the colonies with black spot. Take five colonies and inoculate each to biochemical media: Triple Sugar Iron Agar (OXOID®), Simons Citrate Agar (OXOID®), Sulfide Indol Motility (BD), and Methyl-Red Voges-Proskauer (OXOID®), incubate for 24-48 hours at 37°C, then confirmation the positive *Salmonella* sp isolates. Purify the positive *Salmonella* sp using Nutrient Agar (Merck®).

Table 1. List of Wet Market in Center of Surabaya and Total Specimens

No	Wet Market	Spesimens
1	Pasar Kembang	5 specimens
2	Pasar Keputran	5 specimens
3	Pasar Dukuh Kupang	4 specimens
4	Pasar Kupang	4 specimens
5	Pasar Pandegiling	4 specimens
6	Pasar Kedungsari	4 specimens
7	Pasar Kedungdoro	4 specimens
	Total	30 specimens

Each of purified positive *Salmonella* sp from Nutrient Agar were sub-cultured to PZ sterile and the turbidity of the isolates equivalent to 0.5 McFarland. Susceptibility of isolates to selected antibiotics was carried out using the Kirby Bauer's disk diffusion method on Mueller-Hinton Agar (BD).[9] Susceptibility to the following antibiotics was determined: Ampicillin-Sulbactam 10 µg (OXOID®), Amikacin 30 µg (OXOID®), Meropenem 10 µg (OXOID®), Ofloxacin 1 µg (OXOID®), Nalidixic Acid 30 µg (OXOID®).

RESULT AND DISCUSSION

Any colonies that grow on Salmonella Shigella order taken five colonies were selected for the best. Then conducted to biochemical tests on Triple Sugar Iron Agar, Simon Citrate, Indol Motility Sulfide and Methyl Red-Voges Proskauer. Out of a total of 30 specimens examined, 27 (90%) were positive for *Salmonella* sp (Table 2).

Contamination could happen when processing on Poultry Slaughter House until the meats were consumed. The contaminants are soil contamination, dirt, water, processing equipment, air, human.[10]

Samples with positive results and then tested again to see the level of sensitivity to antibiotics. This sensitivity test using Kirby-Bauer method and the antibiotics that used are a class of β-Lactam antibiotics (ampicillin sulbactam), a sub-class of carbapenems (meropenem), aminoglycosides (amikacin), fluoroquinolones (ofloxacin), and quinolones (nalidixic acid).

High percentages of theisolates were susceptible to Meropenem and Amikacin (100%), Ampicillin Sulbactamand Ofloxacin (88.9%). However, 44,4% isolates were resistant to Nalidixic Acid.

Mechanism of antibiotic resistance could be transferred via plasmid (R factor), a genetic mutation of bacteria that could change the location of binding sites of antibiotics, bacterial metabolic change so that is not affected by antibiotics, or the change of bacteria cell membrane permeability and its difficult to be penetrated by antibiotics.[11,12]

Table 2. *Salmonella* sp Contamination on Broiler Meat at Wet Market in Center of Surabaya

No	Wet Market	Number of Specimen	Positive Salmonella sp	
			Total	Proportion
1	Ps. Kembang	5	3	60%
2	Ps. Kupang	4	3	75%
3	Ps. Dukuh Kupang	4	4	100%
4	Ps.Pandegiling	4	4	100%
5	Ps. Kedungsari	4	4	100%
6	Ps. Kedungdoro	4	4	100%
7	Ps. Keputran	5	5	100%
Total		30	27	90%

Table 3. Antibiotic Susceptibility pattern of *Salmonella* isolated from Broiler Meat at Wet Market in Center of Surabaya

No	Antibiotics	S[a]	%	I[b]	%	R[c]	%
1	Ampicillin Sulbactam	24	88,9%	2	7,4%	1	3,7%
2	Meropenem	27	100%	0	0%	0	0%
3	Amikacin	27	100%	0	0%	0	0%
4	Ofloxacin	24	88,9%	0	0%	3	11,1%
5	Nalidixic Acid	13	48,2%	2	7,4%	12	44,4%

[a] Sensitive, [b] Intermediate, [c] Resistant

Meropenem has a good result, 27 samples was susceptible to *Salmonella* sp. Meropenem is antibiotic that could be the final choice for treating the Gram-negative bacteria infection. From Center of Disease Control and Prevention (CDC) report on Antibiotics Resistance Threat in the United States 2013, Antibiotic resistance of carbapenems sub-category could be found on Gram-negative bacteria, included Pseudomonas and *Acinetobacter* spp. After the bacteria became resistant to carbapenems, the bacteria normally resistant to all β-laktam antibiotics. Amikacin resistance occured due to the expression of the gene encoding β-lactamase. This gene encodes the enzyme β-lactamase that inactivates β-lactam ring of Amikacin, therefore becoming resistant to Amikacin.[13]

Amikacin is a good antibiotic for *Salmonella* sp, 100% samples were positive sensitive to this antibiotic. Amikacin is one of semi synthetic aminoglycoside antibiotic that is highly resistant to enzymes modification. Resistance may occur because of three things, The decline retrieval; the absence of oxygen-dependent transport system for aminoglycosides, Lack of receptor; 30s ribosomal sub-unit has a low affinity for aminoglycosides, Modification of the enzyme; plasmids that carry R.factor which encodes an enzyme formation (example: acetyl transferase, nucleotidyl transferase and phosphotransferase) change and inactivation of aminoglycosides antibiotics.[14]

Antibiotic ampicillin-sulbactam has only one positive isolates resistant. Two samples including intermediates to antibiotics and the rest of 88.9% is still sensitive samples. The occurrence of resistance to ampicillin-sulbactam due to the expression of the gene, i.e. the gene encoding β-lactamase is located on Gram-negative bacteria chromosome. This gene encodes the enzyme β-lactamase that inactivates β-lactam ring of ampicillin by means of hydrolyzing β-lactam ring, thereby becoming resistant to ampicillin.[15]

Antibiotic sensitivity of ofloxacin is 88.9% of isolates of *Salmonella* sp still sensitive to these antibiotics. Three isolates were resistant or 11.1%, so it can be said that

Salmonella sp begin resistant to ofloxacin. Ofloxacin is an antibiotic that belongs to the class of fluoroquinolones. The mechanism of resistance to fluoroquinolones is this antibiotic bound to the β subunit of the bacterial enzyme DNA gyrase and block the activity of enzymes that are essential in maintaining DNA supercoiling and important in the process of DNA replication. Mutations in encoding gene of the DNA gyrase could produce active enzyme but could not be bound by fluoroquinolones.[14]

Bacteria *Salmonella* sp has the highest resistance to the antibiotic level Nalidixic acid as many as 12 samples or 44.4% resistant and two samples or 7.4% intermediates. Nalidixic acid is active against Gram-negative bacteria coliform. These antibiotics work by inhibiting the enzyme activity of bacterial DNA gyrase that disrupts DNA supercoiling.[16] Nalidixic acid resistance to antibiotics is not transferred via plasmids (R factor), but by other mechanisms. The mechanism is a genetic mutation of bacteria that can change the location of the protein and binding sites of antibiotics, bacterial metabolic change so it is not affected by antibiotics, or bacteria alter the permeability of the cell membrane so difficult to be penetrated by antibiotics. This resistance has led to clinical problems, bacteria normally resistant to Nalidixic acid is Pseudomonas spp.[11, 12]

CONCLUSION

From the result of this study, Broiler meat sold in wet markets Surabaya center 90% positive contaminated with *Salmonella* sp (27 of 30 samples). *Salmonella* sp isolated from Broiler meat sold in wet markets Surabaya center were 0% resistant to Meropenem and Amikacin, Ampicillin Sulbactam (3.7%), Ofloxacin (11.1%), and Nalidixic Acid (44.4%).

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