Conference Paper

Detection of Antibiotic Residues and Concentration in Raw Milk from Lembang Small Holder Dairy Farm

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Abstract

The study of "Detection of Antibiotic Residues and Concentration in Raw Milk from Lembang Small Holder Dairy Farms" was held in 3 Tempat Pelayanan Koperasi (TPK) or Cooperative Service Center, members of Koperasi Peternak Sapi Bandung Utara (KPSBU) or Dairy Farmer Cooperative of North Bandung, in Lembang, Kabupaten Bandung, which was chosen purposively, and Unit Pelayanan Teknis Sumber Daya Alam Hayati (UPT SDA Hayati) laboratory in Padjadjaran University at Jatinangor, Sumedang. The subject of this study are to detect qualitative and quantitative possibility of antibiotic presence in raw milk from small holder dairy farms members of KPSBU Lembang.

The first step of qualitative antibiotic residues detection was done with a set of Beta Star 25 which taken directly after milking. The positive samples obtain from the first stage test, continued with another test using the same method for 3 consecutive days and second stage quantitative test with microbiological method (Four Plate Test or Frontier Post Test) using *Bacillus subtilis* as indicator bacteria. The data obtained from the study was analyzed descriptively, as given in the results below:

(1) The first stage showed 5 from 18 samples taken (27.78%) was positive. The continuous test from the positive samples showed positive results from day one to day three. The fact indicated presence of antibiotic residues in the raw milk.

(2) The result of the second stage test showed presence of antibiotic residues in the raw milk sample from first day until third day of the study. Some of it had been qualified according to the SNI 2000 regulation. The concentration of residues on the first day, second day and third day average in pH 6.0 were 19.60± 4.62; 9.80± 5.22; and 7.00± 5.20; while in pH 8.0 were 10.40±4.34; 9.00±3.81; and 1.40±0.55

Keywords: Residues, Antibiotic, Raw milk, Residues Concentration, Small Holder Dairy Farms.

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1. Introduction

The increase needs of safety and high quality milk only achieved by good handling management of dairy cows. High quality milk standards in Indonesia requires physical and chemical standards according to Surat Keputusan Direktur Jenderal Peternakan No.17/Kpts/DJP/DEPTAN/1983. Based on Board of Food and Drug Safety Watch (Badan Pengawasan Obat dan Makanan (BPOM), 2003), safety milk means the milk does not contain any substances that cause any health problems or toxicity for consumers. To reach certain standards, food producers need to obey food safety procedures because consumers demand higher safety food standards.

Food safety aspects means the products does not contain contagious disease, any harmful substances or residual compound that endanger human's health, free from biological and chemical substances, pesticides, heavy lead, antibiotics, hormones, drugs and contagious microorganism. This aspects can be reach by comprehensive quality control from animal cultivation techniques, nutrition, health management system, processing, crop handling, storage and product distribution (Kesmavet, 1996). Effective control measures from pre-production until post production needed to prohibit any health problem derived from food consumption or foodborne disease. Food safety is an output of interaction between microbiological toxicity, chemical toxicity, nutritional status and consumer satisfaction. Those standards can be achieved by comprehensive and continuous supervision according to Hazard Analysis Critical Control Point (HACCP) in all chain management process from pre-production, main process, post production, cultivation process, storage, distribution until the product utilized and consumed by consumers (Badan POM 2003., Snyder, 1992).

Disease in milk cattle such as mastitis, enteritis, dermatitis and other disease made the use of antibiotics for curing the animal cannot be avoided because healthy milk only produced by healthy cow (Murdiati, 1997, SNI 2000). Uses of antibiotics in healing the cattle must meet some requirements: (1) Curing diseases, (2) Antibiotics dosage uses suitable according the drug factory regulation, (3) Antibiotic residue in milk less than maximum standards according to SNI 2000; Tetracycline 0,05 mg/kg, Penicillin 0,1 mg/kg and Streptomycin 0,1 mg/kg, (4) Fulfill drug withdrawal time. 2-5 days after antibiotics treatment, the milk obtained should not be distributed to the Koperasi (Kusumaningsih *et al*., 1996).

Antibiotics kill unwanted bacteria in cattle in order to have healthier cattle, undisturbed growth, controlling reproduction, improve cattle quality and feed efficiency (Murdiati, 1997). Thus, uses of antibiotics may enlarge antibiotics residue presence

in milk products (Sudarwanto, 1990., Murdiati and Bahri, 1991 cited by Murdiati, 1997). Antibiotics residues presence in milk may cause allergic reaction, poisoning, failed antibiotic treatments because of bacteria resistance to antibiotics, carcinogenic and unbalanced micro flora count in digestion system (Murdiati, 1997). According to research by Murdiati *et al*. (1995), in raw milk derived from 5 big cities in West Java (Bandung, Bogor, Cianjur, Sumedang dan Sukabumi) was found containing Tetracycline and Penicillin antibiotics residues. Antibiotic residues in raw milk occurred because the farmers did not have sufficient information about withdrawal time after antibiotic treatments.

Among antibiotics, Tetracycline was one of the widely used antibiotic in cattle treatments. Broad spectrum antibiotic, effective in gram positive and negative bacteria, works as bacteriostatic that prohibit microorganism growth. Beside Tetracycline, Penicillin were also used. This bactericidal substances reacted and kill bacteria (Sukadana, 1998). According to Oka *et al*. (1995). Tetracycline and Penicillin most effective reaction was in Frontier Post Test or Bogart's' and Wolf's Four Plate Test (FPT) in test plate contain *Bacillus subtilis* pH 6.0. While Aminoglycoside antibiotics such as Streptomycin which works as bactericidal, effective in test plate contain *Bacillus subtilis* pH 8.0.

The aim of this research was to detect antibiotic residues presence in raw milk from small holder dairy farms members of Koperasi Peternak Sapi Bandung Utara (KPSBU) or Dairy Farmer Cooperative of North Bandung in Lembang (qualitative) and concentration of residues (quantitative) using microbiological methods. Data derived from this study give practical information about the importance of withdrawal time after antibiotic treatments and possibility of danger behind unwanted antibiotic residues for the consumers. This research was held in 18 small holder dairy farm members of 3 Tempat Pelayanan Koperasi (TPK) or Community Service Center, members of KPSBU Lembang, Kabupaten Bandung, which was chosen purposively, and Unit Pelayanan Teknis Sumber Daya Alam Hayati (UPT SDA Hayati) laboratory in Padjadjaran University at Jatinangor, Sumedang.

2. Material and Methods

The study of milk safety from small holder dairy farms was conducted in two stages:

2.1. First Stage

2.1.1. Materials

Raw milk samples for the first stage test uses 18 samples derived from 3 out of 22 TPK which was chosen purposively. From 3 TPK, selected 6 members of Tempat Penampungan Susu (TPS) or Milk Collecting Center as sample respondents (Sokal, 1992). Then from 18 samples collected, took 10 cc of raw milk samples right after milking and tested using Beta Star 25 (Prosedur Mutu PT. ISAM, 2003). Tools:

1. Beta Star 25, antibiotic residue strip tester,
2. Plastic bag, as milk samples storage, (3) Cooler Box, contain ice cube to maintain temperature delivery process from the farmers to the lab (<4°C),
3. Thermometer, for controlling temperature (5) Water bath, for Beta Star 25 test.

2.1.2. Methods

1. In preliminary test, conduct a survey to get exact amount of milk cow and lactation cow in dairy farm members of KPSBU Lembang. 18 raw milk samples from TPS tested qualitatively using Beta Star 25 to detect antibiotic presence.
2. Put milk sample inside plastic bag. Store in cooler box. Control milk delivery from farmers to laboratory less than 4°C using thermometer.
3. Test raw milk samples using Beta Star 25. Take out Beta Star 25 box from the fridge, use one small bottle contain receptor.
4. Open aluminum and rubber cover, put disposable tips to the syringe and milk samples 2cm, push the syringe, put it back carefully.
5. Put milk sample to the small bottle contain receptor, stir until mixed completely.
6. Put bottles of samples in the water bath 47.5°C±0.5°C for 3 minutes.
7. Put dipstick inside the bottle for 2 minutes in 47.5°C.
8. After 2 minutes, see the dipstick results.
9. Dipstick saved as reference file.

2.1.3. Results interpretation

1. Antibiotic test considered not valid if there was no red line in Beta Star 25.

2. If the first line thicker that the reference line, samples considered negative or not containing antibiotic residues.

3. If the first line intensity same or lower than the reference line, sample considered positive.

4. If the first line did not present, sample considered positive (Prosedur Mutu PT ISAM).

5. If the sample shows positive results, continue with next stage test. Positive or negative results in the test considered as antibiotics presence in raw milk samples. Data derived from the first stage test analyzes using descriptive method and considered as qualitative test.

2.2. Second Stage

2.2.1. Materials

(1) Raw milk that show positive results in the first stage, (2) Positive milk samples in the first stage tested in Laboratorium UPT SDA Hayati Universitas Padjadjaran Jatinangor, using Nutrient Agar Plate:

(a) pH 6.0 for Penicillin and Tetracycline, (b) pH 8.0 for Aminoglycosides such as Streptomycin, (3) *Bacillus subtilis* bacteria, in pH 6.0. Penicillin and Tetracycline sensitive, (4) *Bacillus subtilis* bacteria, in pH 8.0 sensitive to Aminoglycoside antibiotics like Streptomycin as indicator, (5) Incubator, (6) Thermometer, (7) pH Meter, (8) Petri dish, (9) Oculating needle, (10) Durham tube, (11) Reaction tube, (12) Sterilized tweezer, (13) Ruler, (14) Pipette.

2.2.2. Methods

1. Second stage test start with making antibiotic residue control (Tetracycline, Penicillin dan Streptomycin). Free of residue Milk added with Tetracycline, Penicillin and Streptomycin antibiotics (dosage 0.05; 0.1; 0.5 dan 1 mg/kg) put inside Durham tubes and flipped over. Then, inserted to Nutrient Agar Plate occulated with *Bacillus subtilis* bacteria in petri dish until it formed milk and antibiotic solution ditch. Petri dish incubated for 24 hours. Keep the petri dish save from flipped over. Results used as indicator.

2. Samples which shows positive results in first stage test using Beta 25, traced to the farmers until 3 consecutive days and continued with Bogaerts' and Wolf's Four Plate Test or Frontier Post Test to find out the possibility of Tetracycline, Penicillin or Streptomycin used (Oka *et al*., 1995).

3. Activate *Bacillus subtilis* gram (-) bacteria in Agar Slant to Nutrient Broth. (a) Use occulating needle, incubated for 24 hours.

   (b) Melt 15 ml Nutrient Agar inside reaction tube, keep it cool until 40°C. Inoculated Nutrient Agar tube with one osse *Bacillus subtilis* gram (-) that was oculated in Nutrient Broth. Condition the Nutrient Agar tube in pH 6.0 dan pH 8.0. Put inside sterilized petri dish until the occulted Nutrient Agar became solid. (c) Put milk samples in Durham Tubes, flipped upside down. Then, inserted to Nutrient Agar Plate occulated with *Bacillus subtilis* bacteria in petri dish until it formed milk ditch.

   Incubated for 24 hours in 30°C. (d) After incubation, measure inhibition zone diameter under the petri dish.

4. Compare inhibition zone with the control petri dish to see the possibility of antibiotic residue concentration, includes Tetracycline, Penicillin dan Streptomycin (mg/kg). Analyze the Data received from this study descriptively.

3. Results and Discussion

First Stage test result from 18 milk samples purposively chosen from 3 TPK members of KPSBU Lembang using Beta Star 25 shows:

First stage test using Beta Star 25 in Table 1, shows 5 from 18 samples positively contain antibiotic residues (27.78%). Samples that shows positive results given code A, B, C, D and E. The milk which shows positive samples traced to the milk farm and continued with second stage test for 3 consecutive days.

Second step in first stage test in Table 2 shows after 3 days of milking, antibiotics residues still presence in raw milk samples. This fact indicated raw milk samples contain antibiotic residues and have not completed antibiotic withdrawal time. For safety reason, milk derived from antibiotic treated cattle should not be consumed within 3 days after last treatments. This hypothesis match with Ressang dan Nasution (1963) and Kusumaningsih *et al*. (1996) statements.

Antibiotic withdrawal time varied within 2-30 days, depends on: 1) Type of drug, 2) Species, 3) Genetic factors of cattle, 4) Local climate, 5) Way of treatment, 6) Dosage, 7) Health status of the cattle, 8) Type of animal products, 9) Drug residue tolerance, 10)

Table 1: First Stage Antibiotic Residue Test.

No	Milk Sample Code	Beta Star 25 Result (+/-)
1	1	-
2	2	-
3	3	-
4	4	+
5	5	-
6	6	-
7	7	+
8	8	-
9	9	+
10	10	-
11	11	-
12	12	-
13	13	-
14	14	+
15	15	-
16	16	-
17	17	+
18	18	-

[a]+: positive samples, contain antibiotic residue

[b]-: negative samples, not containing antibiotic residue

Table 2: Second Step in First Stage Test.

No	Milk Sample Code	Test Result		
		Day 1	Day 2	Day 3
1	A	+	+	+
2	B	+	+	+
3	C	+	+	+
4	D	+	+	+
5	E	+	+	+

Drug formulation. In spite of that, not all drug factory communicate withdrawal time in the packaging.

Table 3: Second Stage Test: Antibiotic Residue in Acid Condition.

No	Milk Sample Code	Inhibition Zone Diameter (mm)		
		Day 1	Day 2	Day 3
1	A	17	**6**	4
2	B	**14**	12	**3**
3	C	**26**	**18**	**16**
4	D	22	7	6
5	E	19	**6**	6
	Average	19,60±4.62	9,80±5.22	7,00±5.20

Table 4: Antibiotic Residue Test Control in Acid Condition.

Residue Control Concentration (gram)	Inhibition Zone Diameter (mm)		
	Penstrep	Vet-oxy	Cloxalak
	(Penicillin)	(Oxytetracycline)	(Penicillin)
0,05	12	10	8
0,1	13	12	10
0,5	14	13	12
1	15	14	14

3.1. Microbiological Methods in Antibiotic Residue Test (FPT)

Second stage antibiotic residue test held quantitatively using microbiological methods also known as Frontier Post Test or Four Plate Test (FPT) to 5 milk samples that shows positive result in first stage test. First, second and third day milk samples tested using *Bacillus subtilis* bacteria occulated in acid condition pH 6.0 to test Penicillin and Tetracycline antibiotic residue. While pH 8.0 or bases uses to test Streptomycin residue (Oka, 1995).

3.2. Antibiotic Residue Test in Acid Condition

Before second stage test in laboratory, researcher made control test using antibiotic drugs that widely use in farm. Control test function as comparison tools for experiment test results.

Data from Table 3 shows acid condition in pH 6.0 suitable for Penicillin and Tetracycline antibiotic residue test. In the first day of the study, inhibition zone ranged from

14 mm until 26 mm. Compared to the control plate, antibiotic residue concentration possibility more than 1 mg/kg in every milk samples.

Except in milk sample B, with inhibition zone diameter 14 mm. Other samples varied from 16 until 22 mm. In first day of the study, lowest inhibition zone reach by milk sample B.

If Penstrep used in the antibiotic treatment, the possibility of antibiotic residue in raw milk was 0.5 mg/kg. If Vet-oxy or Cloxalak used in the treatment the possibility of antibiotic residue in raw milk was 1 mg/kg. Average of the first day test in acid condition was 19.60+4.62.

Second day test in acid condition show inhibition zone diameter range between 6 mm until 18 mm. Over all inhibition diameter zone in day 2 declined compared to first day of the study. Largest inhibition zone diameter was 18 mm, found in sample C. This result indicate antibiotic residue concentration in raw milk samples more than 1 mg/kg for every antibiotics might be used for the treatments. Though inhibition zone diameter decline 8 mm from 26 mm in first day.

Lowest inhibition zone diameter 6 mm reach by sample A and E, 7 mm by sample D indicate antibiotic residue in raw milk less than 0.05 mg/kg and qualified according the standards approved by Indonesian National Standards (Standar Nasional Indonesia 2000). But varied results shown in second day of the test, reflected that not all samples approved by SNI 2000 standards and should not be consumed for safety reasons. Average of day 2 results in acid condition was 9.80+5.22.

Third day test result in acid condition shows inhibition zone diameter ranged between 3 mm until 16 mm. Except sample C result which reach 16 mm, others ranged between 3 mm until 6 mm. This results shows most of the milk samples qualified for antibiotic residue concentration according to SNI 2000, which stated that antibiotic residue must be less than 0.05 mg/kg. Sample C which had 16 mm inhibition zone diameter reflected that antibiotic residue concentration in raw milk more than 1 mg/kg for every types of antibiotics. Average of third day test in acid condition was 7.00+5.20.

Although almost all of the raw milk samples qualified for SNI 2000 (except sample C), consumers safety will be more guaranteed if raw milk was not consumed 3 days after withdrawal time.

3.3. Antibiotic Residue Test in Bases Condition

Second stage test in bases condition pH 8.0 held to detect antibiotic residue Amino-glycoside (Streptomycin) and Penicillin (Ampicillin) type compared with control plate.

Table 5: Second Stage Test: Antibiotic Residue in Bases Condition.

No	Milk Sample Code	Inhibition Zone Diameter (mm)		
		Day 1	Day 2	Day 3
1	A	**16**	**13**	**2**
2	B	**4**	**3**	**1**
3	C	10	8	**1**
4	D	10	10	**2**
5	E	12	11	**1**
	Average	10.40± 4.34	9,00± 3.81	1.40± 0.55

Table 6: Antibiotic Residue Test Control in Bases Condition.

Residue Control Concentration (gram)	Inhibition Zone Diameter (mm)		
	Penstrep (Penicillin)	Vet-oxy (Oxytetracycline)	Cloxalak (Penicillin)
0,05	9	9	**8**
0,05	9	9	8
0,1	11	10	9
0,5	12	12	12
1	13	13	14

First day test result show inhibition zone diameter ranged between 4 mm until 16 mm. Sample milk B which shows 4 mm had met the criteria approved by SNI 2000 in all type of antibiotics. Average of first day results in bases condition was 10.40±4.34.

Second day test in bases condition show inhibition zone diameter ranged between 3 until 13 mm. Sample B inhibition zone diameter was 3 mm, indicated this sample qualified for standards according to SNI 2000 in any kind of antibiotic used for control plate.

While other samples had varied results from 0.05 mg/kg until 1 mg/kg concentration. There was antibiotic residue decline between first to second days of the test in bases condition. Except D milk sample, antibiotic inhibition zone remain 10 mm from first to second day of the test.

Second day test average in bases condition was 9.00±3.81. Most of the milk residues in second day of bases condition was in maximum limit qualified by SNI 2000. But not all the samples suitable with SNI 2000 regulation. In order to maintain safety standards, raw milk from cattle that treated with antibiotic should not be consumed or marketed within 2 days after treatment.

Antibiotic residue in the third day of the test shows inhibition zone diameter ranged between 1 mm until 2 mm in all samples. Third day average in bases condition was 1.40+0.55. Antibiotic residues in all samples had major decline compared to first and second day of the test and qualified according to SNI 2000. But if acid condition results in the third day combine with bases condition, raw milk samples had not reach safety standards. For safety reason, raw milk should not be consumed within three days after last day of antibiotic treatment. Decrease of antibiotic concentration in raw milk samples from first day until third day after last day of antibiotic treatments happen because milk cattle continue to produce milk. Like dilution process in fluid substances, if the solvent added continuously to the solution the concentration of dissolved substances will continue to decrease.

According to the interviews held during the study, main factors of antibiotics residues presence in raw milk from small holder dairy farms was lack of information and socialization of withdrawal time after antibiotic treatments. The same results shows in similar study held by Kusumaningsih *et al.*, in 1996. Farmers did not know type of drugs or antibiotics given during the treatments, including withdrawal time information. They continued selling the raw milk 2-5 days after antibiotic treatments. Economic reason was also the main factor of antibiotic residue presence. If the farmers did not sell the raw milk to the Koperasi, their income would reduce. Moreover, Koperasi did not have drug residue criteria in their standards, so there was no reason to reject milk if other standards approved.

Antibiotic residues presence in milk may cause allergic reaction, poisoning and antibiotic resistance. Whereas Streptomycin could not disappeared in heating process. Another impact of antibiotic residue presence cause milk product from Indonesia hard to compete in the free trade market. One of the requirement standards was free of residue products (Murdiati, 1997).

Importance of socialization on drugs from the health inspector to the dairy farmers especially antibiotics withdrawal time during milk cattle health treatment is necessary. Most of all, dairy farmers awareness on withdrawal time in obeying safety standards.

4. Conclusion

First stage antibiotic residue test in qualitative method shows 5 from 18 (27.78%) milk samples were positive. Continuous test from first, second until third day show the same results. This results indicated antibiotic residue presence in raw milk samples had not pass withdrawal time. Second stage antibiotic residue test in quantitative methods

using microbiological test shows antibiotic residue in several milk samples qualified according to SNI 2000. But there were still samples that not pass safety milk standards according to SNI 2000. First, second and third day antibiotic residue average in pH 6.0 were: 19.60±4.62; 9.80±5.22; and 7.00±5.20; while in pH 8.0 were 10.40±4.34; 9.00±3.81; and 1.40±0.55 According to the test results, socialization to dairy farmers on withdrawal time and time limit when milk free of antibiotic residues can be distributed to consumers was important. Dairy farmer awareness regarding withdrawal time was also important in order to reach safety aspect in raw milk products.

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