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# ANTIBIOTIC RESISTANCE MICROORGANISMS IN RAW MILK AND IMPLEMENTATION OF SANITATION STANDARD OPERATING PROCEDURE (SSOP) DURING MILKING PROCESS

**Souvia RAHIMAH*, Imas Siti SETIASIH*, Roostita L. BALIA**, Roni KASTAMAN***

Padjadjaran University, *Faculty of Agriculture Industrial Technology, **Faculty of Animal Husbandry, Indonesia, Email: souvia@unpad.ac.id

***Corresponding author***: souvia@unpad.ac.id

***Abstract***

*The spread of antibiotic resistance microorganisms is currently one of the most important safety issues including raw milk and milk products. Contamination of microbes occurs during milking, shortly after milking until the milk is consumed. Contamination could come from the environment, workers, equipment, cages, animals, feed and water. To reduce the risk of microbial contamination application of Sanitation Standard Operating Procedure (SSOP) during milking process is absolutely necessary. The aims of this study were to determine antimicrobial resistance of Staphylococcus aureus, Escherichia coli and Salmonella isolated from raw milk and the implementation of SSOP during milking process. The results showed that with the average level of SSOP implementation in milking process of 61.85% or 20 SSOP, the antimicrobial resistance qualitative examination were found that the milk samples consisted of E. coli, Staphylococcus aureus and Salmonella. The antibiotic resistance profiles were tested to 3 antibiotics. It showed that 44.44% E. coli, 22.22% Staphylococcus aureus, and 11.11% Salmonella were resistance to chloramphenicol, where as all bacteria (100%) were resistance to trimethoprim and 11.11% E. coli were resistance to ampicillin.*

***Key words**: bacteria, antibiotics, resistance, milk*

## INTRODUCTION

Milk is one of the most important food commodities in the world because it contains important elements that are balanced and easily digested to build and nourish the body. Almost all races in the world consume milk [2]. Milk contains high-grade protein, consisting of 18 percent whey protein and 82 percent casein containing essential amino acids. Milk contains minerals such as calcium, phosphorus, magnesium, and potassium.

The production of domestic fresh milk and milk products is still constrained on the production capacity, sustainability and quality of milk produced. To produce quality milk and safe to consume, good handling and managemant is needed from the beginning of milk production.

Contamination occurs shortly after milking until the milk is consumed. Contamination can come from outside the udder or the environment such as workers, equipment, cages, animals, feed and water. The quality management of fresh milk production is absolutely necessary to reduce the risk of microbial contamination and growth in milk. Good Dairy Farming Practices, immediate post-dairy handling, and good distribution should be applied to produce fresh, quality and safe milk. Good Dairy Farming Practices (GDFP) is the first step in quality management and safety of milk and dairy products at farmer level. The quality and safety management program is the implementation of the milking sanitation procedure known as Sanitation Standard Operating Procedures (SSOP) milking process. GDFP and SSOP this milking process is a prerequisite when someday companies want to obtain the Hazard Analysis Critical Control Point (HACCP) certificate.

The quality of fresh milk must meet the standards set in Indonesia, which is based on SNI No. 01-3141-2011 regarding the Quality Standard of Fresh Milk [13]. One of the characteristics of fresh milk that plays an important role is contamination of microbes and contaminants in addition to other contaminants contained in it, such as antibiotics and mycotoxins.

Some bacteria have a natural ability to resist with antibiotics even if they do not interact directly. This can happen because bacteria have enzymes that can inhibit antibiotic work [5]. The usual antibiotics given to cows include oxytetracycline, ampicillin, penicillin (a class of penicillin antibiotics); penstrep (combination of penicillin and cephalosphorin) and sulfadiazine group antibiotics. Bacteria that are resistant to antibiotics are thought to contribute to the high content of microbial contamination in milk.

The aims of this study were to determine and antimicrobial resistance of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* isolated from raw milk and correlation the presence of those bacterias with SSOP application during milking process which can then be identified factors that cause the degradation of the quality of fresh milk.

## MATERIALS AND METHODS

This study consists of 3 stages:

*(i)Identify the implementation of SSOP of milking process by dairy farmers*. A total of 97 respondents of milk cooperative members were interviewed to find out the application level of 33 SSOP variables that were built based on cooperative milking SOP and FDA (2015) [7].

The 33 variables of milking process SSOP are:

(1)Milk from a sick animal is always separated
(2)Throw 3 - 4 first milk juice
(3)Clean the cage from the dirt
(4)Filter the milk to be put into the milk can
(5)Bring milk immediately to the shelter after dairy
(6)The cage has a good lighting system, day and night
(7)There is a good drainage channel
(80Drying hands after washing hands with a clean cloth
(9)Milking in the right way
(10)Cages have good air circulation
(11)The enclosure enclosure is made of a material that is easy to clean and not slippery
(12)Cook water until it boils to clean the milking apparatus and wipe the udder
(13)Stainless steel or aluminum container bucket
(14)Wiping the udder dry
(15)Workers are always ascertained in good health
(16)Separate cages with other animal enclosures
(17)Clean the udder with warm water before milking
(18)The water used meets the criteria of clean water
(19)Cleaning the milking equipment with soap
(20)Special place of storage of appliances and in clean condition
(21)Rinse the milking tools with hot water
(22)Bathing the cow before dairy
(23)Tie the cow's tail when milked
(24)Hand washing using soap before dairy
(25)The feeding area is separate from the enclosure
(26)Dipping the nipple into the iodine solution after milking
(27)Clean cage roof
(28)There is a special room for storing tools
(29)Drying the milking equipment by drying
(30)Using clean clothes during dairy
(31)Having a litter bin
(32)Separate milking room and cages.
(33)Cool down immediately after milking up to 4 $^{o}$C.

*(ii) Escherichia coli, Staphilococcus aureus and Salmonella isolation*

Samples of milk was taken from 9 dairy farmers, collected and cooled immadiately at 4$^{o}$C and brought to the laboratory..

<u>*E. coli*</u>

Each sample was enriched in pre-enrichment buffer peptone water (BPW) and incubate at 38$^{o}$C for 48 hours. Each innoculum was streak on Eosin Methilene Blue (EMB).

<u>*S. aureus*</u>

Each sample was enriched in pre-enrichment buffer peptone water (BPW) and incubate at 38$^{o}$C for 48 hours. 0,1 ml suspension was inoculated on Baird Parker Agar at 37°C for 48 hours.

Salmonella spp

Each sample was enriched in pre-enrichment buffer peptone water (BPW) and incubate at 38°C for 48 hours. Bacteria suspension was innoculated in Xylose Lysine Desoxycholate (XLD).

*(iii)Antibiotic resistance analysis*

Each bacterial colony was tested for resistance to chloramphenicol antibiotics, trimethroprim and tetracycline. Determination of antibiotic doses was performed on the basis of MIC (minimal inhibitory concentration). Based on the results of the Soleha (2015) [18] study, MICs for chloramphenicol, tetrsycline and trimethoprim were 30μg, 30μg, and 5μg, respectively. Testing of microbial resistance to antibiotics is done by antibiotic resistance test by method so that the diffusion is done by way of wells [1] [14].

In the diffusion method, the media used is Mueller Hinton and the method used is the way of the well. The $10^8$CFU / ml bacterial suspension is flattened on agar medium, then for a particular center line to be made according to need. The antibiotic solution used is dripped into the well. Incubated at 37°C for 18-24 hours. Read the results, as in the Kirby-Bauer way [15].

## RESULTS AND DISCUSSIONS

The results of the implementation of SSOP can be presented in Table 1 which shows that not all SSOPs are implemented by all farmers, the highest value is SSOP "Milk originating from sick animals always separated" is implemented by 84 respondents or 86.6%. None of the respondents had a milking room separate from the milking parlor and that cooled the milk directly after milking up to 4°C. Both of these SSOP variables are critical points of microbial contamination in milk.

Table 1 shows that 6 samples from 9 tested samples (66.67%) positive contain *E. coli* ie samples A, B, C, E, H, and I. Three (3) samples originating from breeders with application of SSOP process milking "Low" entirely positive contains E. coli.

Each of two samples from breeders with SSOP application of "Intermediate" and "Low" positive milking processes contain *E. coli*. This means that all levels of SSOP implementation are polluted by *E. coli*.

Table 1. Percentage of SSOP Implementation of Milking Process by Farmers (%)

| No | Farmers Who Implemented SSOP (People) | Percentage of Farmers Who Implement SSOP (%) |
|---|---|---|
| 1 | 84 | 86.6 |
| 2 | 84 | 86.6 |
| 3 | 82 | 84.5 |
| 4 | 79 | 81.4 |
| 5 | 76 | 78.4 |
| 6 | 77 | 79.4 |
| 7 | 74 | 76.3 |
| 8 | 75 | 77.3 |
| 9 | 74 | 76.3 |
| 10 | 74 | 76.3 |
| 11 | 72 | 74.2 |
| 12 | 72 | 74.2 |
| 13 | 71 | 73.2 |
| 14 | 72 | 74.2 |
| 15 | 71 | 73.2 |
| 16 | 71 | 73.2 |
| 17 | 69 | 71.1 |
| 18 | 68 | 70.1 |
| 19 | 63 | 64.9 |
| 20 | 62 | 63.9 |
| 21 | 60 | 61.9 |
| 22 | 61 | 62.9 |
| 23 | 60 | 61.9 |
| 24 | 58 | 59.8 |
| 25 | 57 | 58.8 |
| 26 | 53 | 54.6 |
| 27 | 50 | 51.5 |
| 28 | 47 | 48.5 |
| 29 | 38 | 39.2 |
| 30 | 31 | 32 |
| 31 | 25 | 25.8 |
| 32 | 0 | 0 |
| 33 | 0 | 0 |

Source: Own results.

Table 3 shows that 5 samples (55.56%) positive contain *S. aureus*, ie samples B, D, F, and I. This means that all levels of SSOP application of the milking process are polluted by *S. aureus*. Two samples come from breeders who implement SSOP milking process "High" and "Intermediate".

One sample came from a breeder with a "Low" implementation of SSOP milking process.

Table 2. *E. coli* Isolation Result

| Implementation of SSOP | Sample | Replication | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| "High" | A | - | - | - |
| | B | - | - | + |
| | C | - | - | + |
| "Intermediate" | D | - | - | - |
| | E | + | - | + |
| | F | - | - | - |
| "Low" | G | + | + | - |
| | H | + | + | - |
| | I | - | - | + |

Source: Own results.

Tabel 3. *S. aureus* Isolation Result

| Implementation of SSOP | Sample | Replication | | | |
|---|---|---|---|---|---|
| | | $10^{-1}$ | | $10^{-2}$ | |
| "High" | A | - | - | - | - |
| | B | 8 | 4 | - | - |
| | C | - | - | - | - |
| "Intermediate" | D | 1 | 3 | - | - |
| | E | - | - | - | - |
| | F | 4 | - | - | - |
| "Low" | G | - | 13 | - | - |
| | H | - | - | - | - |
| | I | 6 | 19 | - | - |

Source: Own results.

Table 4. shows that 1 sample (11.1%) positively contains Salmonella.

Table 4. Salmonella Isolation Result

| Implementation of SSOP | Sample | Replication | |
|---|---|---|---|
| | | 1 | 2 |
| "High" | A | - | - |
| | B | - | - |
| | C | - | - |
| "Intermediate" | D | - | - |
| | E | - | - |
| | F | + | + |
| "Low" | G | - | - |
| | H | - | - |
| | I | - | - |

Source: Own results.

This is a sample derived from a breeder with the application of "intermediate" SSOP milking process implementation.

After isolation, bacteria were found to be tested for resistance to antibiotics of chloramphenicol (30 μg), tetraskilin (30 μg) and trimethoprim (5 μg). The dose and standard used refers to Soleha (2015) [18] presented in Table 5.

Tabel 5. Standard Diameter Interpretation Zone for Determination of Microbial Sensitivity Criteria against Antibiotics

| **Antibiotics** | **Zone Diameter (mm)** | | |
|---|---|---|---|
| | Resistant | Intermediet | Sensitive |
| Chloramfenikol (30 μg) | <12 | 13 – 17 | > 18 |
| Tetraskilin (30 μg) | <14 | 15 – 18 | >19 |
| Trimetoprim (5 μg) | <10 | 11 – 15 | >16 |

Source: Own results.

Table 6 shows that of from 9 isolates, 4 isolates of *E. coli*, 2 isolates of *S. aureus* and 1 isolate *Salmonella* (77.8%) proved resistant to chloramphenicol. All bacterial isolates are resistant to trimethoprim (100%). Only 1 isolate of E. coli was resistant to ampicillin (11.1%).

All milk samples from breeders applying the SSOP of the "low" positive milking process *contain E. coli* which is resistant to the three types of antibiotics tested. Milk samples from breeders applying the "good" milking SSOP, still contain S. aureus and Salmonella which are resistant to chloramphenicol and trimethoprim.

The overuse of antibiotics in the livestock industry has led to a high diversity of various antibiotic resistance genes (ARGs) found in manure [11] [3].

Animal waste is one of the contaminants in milk. Contamination of impurities containing antibiotic-resistant microbes is also suspected to be propagated through manure [17]. The process of aerobic composting of cattle manure is suspected to be a cause of antibiotic resistance [4].

*Staphylococcus aureus* is one of the causes of mastitis in cows and may contaminate milk throughout its supply chain, causing high milk microbial contamination and possible Staphylococcal food poisoning (SFP) toxicity [16]. The prevalence of milk contamination by *Staphylococcus aureus* is resistant to antibiotics in fresh milk varies, reported 51% to 91% [8] [9].

Table 6. Inhibitory Zone of EC, SA and SAL bacteria against chloramphenicol antibiotics, trimethoprim and tetracycline

| Code | Inhibitory Zone Diameter (mm) | | | | | |
|---|---|---|---|---|---|---|
| | A (chloramphenicol) | | B (trimethoprim) | | C (tetracycline) | |
| | Inhibitory Zone (mm) | Criteria | Inhibitory Zone (mm) | Criteria | Inhibitory Zone (mm) | Criteria |
| EC-A | 9.65 | Resistant | 6.85 | Resistant | 21.80 | Sensitive |
| EC-B | 11.13 | Resistant | 0 | Resistant | 17.00 | Intermediet |
| EC-C | 17.75 | Intermediet | 3.5 | Resistant | 9.50 | Resistant |
| EC-E | 9.50 | Resistant | 0 | Resistant | 25.65 | Intermediet |
| EC-H | 8.88 | Resistant | 0.65 | Resistant | 16.25 | Intermediet |
| SA-B | 14.15 | Intermediet | 0 | Resistant | 16.49 | Intermediet |
| SA-D | 11.00 | Resistant | 0 | Resistant | 19.35 | Sensitive |
| SA-F | 9.65 | Resistant | 0 | Resistant | 15.15 | Intermediet |
| SAL-G | 10.55 | Resistant | 0 | Resistant | 15.65 | Intermediet |

Source: Own results.

*S. aureus* resistant to antibiotics began to emerge several decades ago due to widespread and often inaccurate use of antibiotics and doses in livestock. Despite the limitation of use, both clinically and in food production, the trend of increased antibiotic resistance continues [6] [10] . The sources of *S. aureus* contamination are, among others, the result of dairy farm sites that are too close to animal cages [12]. Survey results show (Table 5) that 29% of breeders place their cages adjacent to other animal enclosures, thus increasing the likelihood of contamination of antibiotic-resistant microbes.

## CONCLUSIONS

The level of implementation of SSOP milking process by breeders is only an average of 61.85% or 20 SSOP. The highest level of SSOP implementation by breeders is 30 SSOP (80%) and lowest 10 SSOP (30.30%).

There are bacteria that are resistant to antibiotics. Of the 9 isolates tested, 4 isolates of *E. coli* (44.44%), 2 isolates of *S. aureus* (22.22%) and 1 isolate *Salmonella* (11.11%) proved resistant to chloramphenicol. All bacteria isolates are resistant to trimethoprim. Only 1 isolate of *E. coli* is resistant to ampicillin.

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