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The Relationship Between Milking Hygiene and Sanitation with Staphylococcus aureus Contamination In Fresh Cow's Milk at Livestock Farmer Groups in Baturaden And Sumbang Subdistricts In 2024

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Abstract

Several studies have shown that contamination of dairy products by pathogenic bacteria such as S. aureus can be influenced by the implementation of hygiene and sanitation practices. However, the majority of traditional dairy farmers are still unable to meet these standards during the milking process. This study aims to analyze the relationship between milking hygiene and sanitation and S. aureus contamination in fresh cow's milk. This research is observational with a cross-sectional design. The research subjects consisted of livestock farmer groups in Baturaden and Sumbang Districts, totaling 30 dairy farmers. Data analysis used the chi-square test and multiple logistic regression, with independent variables including barn sanitation, equipment sanitation, and the personal hygiene of milkers, and the dependent variable being S. aureus contamination in fresh cow's milk. The study found that 18 (60%) did not meet the requirements for barn sanitation, 21 (70%) for equipment sanitation, 18 (60%) for personal hygiene of milkers, and 19 (63.3%) of fresh cow's milk samples. There is a relationship between barn sanitation (p-value 0.009), equipment sanitation (p-value 0.000), and personal hygiene of milkers (p-value 0.000) with S. aureus contamination in fresh cow's milk. The most influential variable is the personal hygiene of the milkers.

Keywords: Milking Hygiene and Sanitation, Staphylococcus aureus, Food safety, Dairy farmer, Fresh Cow's Milk

Introduction

Government Regulation Number 66 of 2014 on Environmental Health explains that, in order to achieve a healthy environmental quality, it is necessary to optimize environmental health management, one of which addresses food as an environmental medium.[1] Food is a biological product derived from agriculture, livestock, and other sources, in the form of raw materials or processed materials that are intended for human consumption.[2] To this day, Indonesia still faces food safety problems, as can be seen from several cases of food poisoning that continue to occur in various regions. The National Agency of Drug and Food Control (Badan POM) recorded 72 cases of food poisoning outbreaks in Indonesia in 2022, with 5 confirmed cases caused by contamination with Staphylococcus aureus and Salmonella.[3] Food products with high nutritional and protein content, such as cow's milk, can be a vulnerable medium for microbial growth.[4] Incidents of poisoning due to milk consumption occurred in 2021 when teachers and kindergarten students at TK Mafatikhul Huda in Tegal Regency consumed raw cow's milk from local farmers.[5] In the same year, there was also a poisoning incident in Cianjur Regency affecting 9 students from SDN Cinanyar after consuming milk purchased from a street vendor near the school.[6] In addition, in 2000, as many as eight prefectures in western Japan experienced an epidemic involving 13,809 cases of foodborne disease caused by three different types of milk that were contaminated with S. aureus during the production line at a milk processing factory in Osaka. The affected victims suffered from diarrhea and vomiting due to enterotoxins produced by S. aureus.[7] Cow's milk can become contaminated with S. aureus during milking or milk handling due to poor hygiene practices. A study conducted at a dairy farm in Jember Regency in 2016 showed a correlation between sanitation hygiene and personal hygiene with the presence of S. aureus in cow's milk. This illustrates the importance of practicing hygiene and sanitation during milking procedures to prevent the risk of microbiological contamination.[8] The rate of milk consumption increases every year in line with the growing population and high protein demand. In 2014, Baturaden District was the top producer of cow's milk, with a total production of 1,801,067 liters, followed by Sumbang District with 200,105 liters.[9] As a tourist area, farmers in Baturaden and Sumbang districts frequently receive requests to supply fresh cow's milk directly to consumers. Preventive measures to minimize the risk factors for microbial contamination in fresh milk can begin at the production stage, namely by implementing good hygiene and sanitation practices during the milking process.[10] However, the majority of traditional small-scale dairy farmers, including those in Baturaden and Sumbang districts, are still unable to meet hygiene

and sanitation standards during these processes. This study aims to determine the relationship between milking hygiene and sanitation practices and S. aureus contamination in fresh cow's milk produced by farmer groups in Baturaden and Sumbang districts.

Materials and methods

Research Design

This research is an analytic observational study with a cross-sectional design. The population consists of Cattle Farmer Group A in Baturaden Subdistrict and Cattle Farmer Group B in Sumbang Subdistrict, with a total of 30 dairy farmers who already represent the entire population.

The independent variables, which consist of barn sanitation, equipment sanitation, and the personal hygiene of the milkers, were assessed using an observation sheet instrument adopted from previous research on the quality of fresh cow's milk in terms of hygiene and sanitation by Prabandari (2021) at dairy farms in Magetan Regency that has been tested for validity and reliability.[11] For a variable to be considered as meeting the requirements, the total score must reach 100%, meaning all assessment components must be fulfilled. The dependent variable, namely S. aureus contamination in fresh cow's milk, was determined through laboratory tests by cultivating the pathogenic bacteria on Mannitol Salt Agar (MSA) selective media at 37°C for 24 hours. The maximum allowable limit for S. aureus contamination in fresh milk refers to SNI 7388:2009, which is 1 x 10^2 colonies/ml.[12]

Univariate analysis was carried out to describe the percentages and frequency distributions of each variable studied. Bivariate analysis was performed using the chi-square test to determine the existence of a relationship between the independent and dependent variables, with the interpretation that the alternative hypothesis (Ha) is accepted if the p-value < 0.05, and rejected if the p-value ≥ 0.05. The strength of the relationship between variables was measured using the correlation coefficient obtained from the contingency coefficient test.[13]

Table 1. Interpretation Guidelines for the Contingency Coefficient Test (Sugiyono, 2013)

Correlation Score	Relationship level
0,00 – 0,199	Very Weak
0,20 – 0,399	Weak
0,40 – 0,599	sufficient
0,60 – 0,799	Strong
0,80 – 1,000	Very Stron

Multivariate analysis was conducted using multiple logistic regression tests to determine which independent variable has the greatest or most dominant influence on the dependent variable. In cross-sectional studies, the interpretation of results can be seen in the magnitude of the relative risk (RR) value. The independent variable with the highest RR value in the final multivariate model becomes the variable that has the most dominant influence on the dependent variable.[14]

Results

The respondents in this study were 30 dairy farmers from Baturaden District and Sumbang District with the following characteristics.

Table 2. Respondent Characteristics

Category	(F)	(%)
Gender		
Male	29	96.7
Female	1	3.3
Age		
< 15 years	0	0.0
15–64 years	27	90.0
≥ 65 years	3	10.0
Length of Employment		
< 5 years	7	23.3
5–15 years	13	43.3
> 15 years	10	33.3
Education Level		
No Schooling	5	16.7
Elementary School Graduate	8	26.7
Junior High School Graduate	8	26.7
Senior High School Graduate	9	30.0

The data in Table 2 show that almost all milkers are male, with a frequency of 96.7%. Ten percent of the milkers are classified as elderly and fall into the non-productive age category, while the remaining 90% are of productive age, ranging from 15 to 64 years old. A total of 43.3% of milkers have worked for 5–15 years, and another 33.3% have been working for more than 15 years. The majority have completed senior high school or its equivalent, with 30% of milkers having attained this level of education.

Tabel 3. Hasil Analisis Univariat Kontaminasi *Staphylococcus aureus* pada Susu Sapi Segar

Kontaminasi *S. aureus*	(F)	(%)
Memenuhi syarat	19	63.3
Tidak memenuhi syarat	11	36.7
Total	30	100

Fresh cow's milk samples collected from respondents were further examined in the laboratory, and the results are shown in Table 3. Based on laboratory tests, it was found that 19 samples (63.3%) did not meet the requirements for the maximum allowable limit of S. aureus contamination in fresh milk, while the remaining 11 samples (36.7%) met the requirements.

Tabel 4. Hasil Analisis Univariat Sanitasi Kandang

Cage Sanitation	(F)	(%)
Meets requirements	18	60
Does not meet requirements	12	40
Total	30	100

The data in Table 4 shows that out of a total of 30 respondents, 60% reported that cage sanitation did not meet the requirements.

Tabel 5. Hasil Analisis Univariat Sanitasi Alat

Tools Sanitation	(F)	(%)
Meets requirements	21	70
Does not meet requirements	9	30
Total	30	100

Data in Table 5 shows that out of a total of 30 respondents, 70% of the equipment sanitation did not meet the requirements. All milk containers are made of waterproof and non-corrosive materials.

Table 6. Results of Univariate Analysis of Milkers' Personal Hygiene

Personal Hygiene	(F)	(%)
Meets requirements	19	63.3
Does not meet requirements	11	36.7
Total	30	100

The data in Table 6 shows that out of a total of 30 respondents, 60% of the milkers' personal hygiene did not meet the requirements. Based on the observations, it was found that all milkers were healthy and were not suffering from skin diseases or easily transmitted illnesses such as coughs, colds, influenza, or diarrhea, and they did not have any open wounds.

Table 7. Contingency Coefficient Test Results

Variabel	Nilai Korelasi
Cage Sanitation	0.453
Tools Sanitation	0.579
Personal Hygiene Pemerah Susu	0.620

The results of the contingency coefficient test in Table 8 show a correlation value of 0.453 for barn sanitation and 0.579 for equipment sanitation, indicating that both variables have a moderate relationship with S. aureus contamination in fresh cow's milk.

Table 8. Bivariate Analysis Results

Variabel	Kontaminasi *S. aureus* TMS (F)	TMS (%)	MS (F)	MS (%)	Total (F)	Total (%)	p-value
Cage Sanitation							
TMS	15	83.3	3	16.7	18	100	0.009*
MS	4	33.3	8	66.7	12	100	
Total	19	63.3	11	36.7	30	100	
Sanitasi Alat							
TMS	18	85.7	3	14.3	21	100	0.000*
MS	1	11.1	8	88.9	9	100	
Total	19	63.3	11	36.7	30	100	
Milkers' Personal Hygiene							
TMS	17	94.4	1	5.6	18	100	0.000*
MS	2	16.7	10	83.3	12	100	
Total	19	63.3	11	36.7	30	100	

Based on the results of the chi-square test in table 8, it can be concluded that there is a relationship between barn sanitation (p-value 0.009), equipment sanitation (p-value 0.000), and the milker's personal hygiene (p-value 0.000) with S. aureus contamination in fresh cow's milk.

Table 9. Results of Multivariate Analysis

Variabel	B	p-value	RR	95% C.I. for EXP(B) Lower	95% C.I. for EXP(B) Upper
Step 1					
Cage Sanitation	2.011	0.192	7.470	0.365	153.095
Tools Sanitation	2.952	0.101	19.138	0.563	650.561
Personal Hygiene milkmaid	3.304	0.024	27.231	1.530	484.567
Step 2					
Sanitasi Alat	2.681	0.081	14.600	0.717	297.484
Personal Hygiene Milkmaid	3.612	0.010	37.036	2.406	570.076

The results of the multiple logistic regression test can be seen in column step 2 presented in table 9. The most dominant variable affecting S. aureus contamination in fresh milk is the personal hygiene of the milker, with a relative risk value of 37.036. This means that if the personal hygiene of the milker does not meet the requirements, there is a 37.036 times higher risk of fresh cow's milk being contaminated by S. aureus compared to when the milker's personal hygiene meets the requirements. Furthermore, the Beta value for the milker's personal hygiene is 3.612, which means that if the personal hygiene of the milker worsens by 1%, there is a 3.612 times higher risk of fresh cow's milk being contaminated by S. aureus.

Discussion

The use of bamboo or wood gets dirty more easily and its construction is not as strong compared to permanent brick walls. Furthermore, there are also slippery barn floors caused by standing water that cannot flow smoothly into the drainage channels. This problem is usually addressed by using carpet or straw as an additional layer for the barn floor. In addition, there are still barns with floors that are not sloped toward the drainage, so liquid waste does not flow directly to disposal and must be removed manually. This can also be a reason for slippery floors and the buildup of waste inside the barn.

Some barns also do not have proper ventilation and air exchange systems due to a lack of openings in the barn or a surrounding environment that is closed off. Poor ventilation can be indicated by stuffy and hot air inside the barn and unpleasant odors due to poor circulation. Most of the barns are mass barns housing many cows separated by partition walls, although a few still consist of just a single communal area for both adult cows and calves. There is one newly built barn, less than 3 months old, which has not yet been equipped with drainage or a trench.

Not all barns have storage facilities for solid or liquid waste. In some cases, solid waste such as cow manure or leftover feed is simply discarded and allowed to accumulate on the outside of the barn, while liquid waste is channeled into drains or rivers. In addition, there are still barns that do not meet the required minimum distance of >10 meters between the barn and the water source. The majority of barns use

municipal water (PDAM), but there are also a few barns that rely on private wells located not far from the barn area as a water source.

All farmers strive to ensure that milk containers are kept clean by washing them before and after milking. Unfortunately, not all farmers wash the milk containers with soap or other specific cleaning agents; some simply rinse the containers with running water before use. Most farmers also do not rinse used milk containers with warm water. This can result in milk fat remaining on the surface of the containers, which can become a breeding ground for microbes. Additionally, some farmers place cleaned milk containers near potential contamination sources, for example by hanging them on barn walls.

Some milkers have slightly long fingernails, making it easy for dirt to get trapped. Not all milkers wash their hands with soap before and after milking; some simply rinse their hands under running water without following proper hand-washing procedures. This can also explain why the hands and fingers of some milkers appear less clean.

Although most milkers already use personal protective equipment in the form of head coverings such as hats, traditional conical hats, tied cloths, or jacket hoods, there are still some milkers who leave their hair uncovered. Additionally, two unhygienic practices were observed: milkers were found smoking while milking and sneezing while handling milk without covering their mouth or nose.

This study demonstrates a relationship between barn sanitation and S. aureus contamination in fresh cow's milk (p-value 0.009). These results are consistent with previous research in Jember Regency, which found that barn cleanliness has a significant association with S. aureus contamination, with a p-value of 0.024. Barns that are full of manure, mud, and leftover feed can increase the risk of transmission and proliferation of S. aureus through parts of the animal's body that come into direct contact with the barn, especially the udder and teats.[15]

Based on another study also conducted in Jember Regency, the analysis showed that the p-value for the condition of the barn floor was 0.011, the p-value for the barn wall condition was 0.013, and the p-value for barn cleanliness was 0.011. This indicates that all three variables have a significant relationship with the presence of S. aureus bacteria in milk. This can occur because dust and dirt on the barn floor, as well as moss on the barn walls, can increase the risk of contamination or pollution of microorganisms in the milk.[8]

Therefore, it is important for farmers to always pay attention to barn sanitation. Implementing barn sanitation is not only related to cleanliness but also to good construction and layout, which maximize the comfort of livestock and minimize the buildup of dirt and residues that can become breeding grounds for bacteria and pathogens. The barn structure should be made permanent so that it is sturdy, durable, and easy to clean. The barn floor should be sloped towards the drainage system to make it easier to dispose of liquid waste and to avoid puddles. The barn floor can also be covered with a carpet made of rubber or with natural straw bedding. It is also important to separate barns for adult cows and calves to reduce the risk of disease transmission from adult cows to more vulnerable calves, as well as to facilitate management of livestock care and health monitoring.[16]

In addition, farmers must consider the placement of water sources, ensuring there is a minimum distance of 10 meters from the barn. A sufficient distance between the barn and the water source will reduce the risk of water contamination by livestock waste, so that the water remains of good quality and safe to be consumed by livestock or used for other activities. Water sources that are close to sources of contamination can increase the risk of water pollution, which will in turn affect the bacteriological quality of the milk produced.[16]

Furthermore, equipment sanitation is also related to S. aureus contamination in fresh cow's milk (p-value 0.000). These research results are in line with previous studies in Jember Regency, which stated that the cleanliness of milk containers has a significant association with S. aureus contamination, with a p-value of 0.024. Repeated use of milk containers without adequate cleaning can increase the risk of contamination by S. aureus. Observations found that during preparation for milking, some farmers did not wash the milk containers thoroughly or only rinsed them with water, assuming the containers were clean enough without the need for washing with soap. Additionally, some farmers still use plastic containers that are quite difficult to clean and do not meet the established standards for milk containers.[15]

S. aureus is a pathogenic bacterium that can rapidly multiply in unhygienic conditions. When milk containers do not meet standards and are not washed properly as recommended, milk residues and other dirt can remain and create an ideal environment for bacterial growth, as leftover milk provides enough nutrients for microorganisms to thrive. In addition, warm and humid environmental conditions can also accelerate bacterial growth.[17]

When this contaminated equipment is reused without adequate cleaning, bacteria can enter freshly collected milk, contaminating it. S. aureus contamination in milk not only reduces the quality and safety of the milk, but also poses serious health risks for consumers. Therefore, proper equipment sanitation is critical to maintaining the microbiological quality of fresh cow's milk.[18]

Farmers are encouraged to use milk containers that comply with standards, such as milk cans or aluminum buckets, which are sturdier and easier to clean compared to thin plastic buckets. Standard milk cans usually have lids that can protect the milk from sunlight, dust, and environmental contamination, thus keeping the milk's quality safe. Farmers should avoid using plastic buckets, as their surfaces are easy to peel and difficult to clean.

The process of washing milk containers must be done using soap or a special cleaning liquid to ensure optimal cleanliness. Washing containers with soap can remove milk fat that remains on the container surfaces,

preventing microbial growth. This procedure should be carried out before and after milking to reduce the risk of contamination. Farmers should also rinse milk containers with warm water after cleaning them with soap. Rinsing with warm water is effective for removing milk fat residues that soap alone cannot erase, thus ensuring the container is completely clean and sterile before reuse.[19]

Milk containers that have been washed must be stored in a clean place, protected from sources of contamination. Farmers are advised to provide a special rack or enclosed storage space, kept far from sources of contamination, to minimize the risk of the milk containers becoming re-contaminated before use.

The personal hygiene of the milkers has a significant relationship with a p-value of 0.000 and has the most dominant influence on S. aureus contamination in fresh cow's milk, with an RR value of 37.036. These research findings are consistent with other studies in dairy farms in Dusun Manggis, Boyolali Regency, which state that the hygiene of milkers has a significant relationship with the total plate count in cow's milk, with a p-value of 0.050. The role of milkers' hygiene is highly crucial to the quality of cow's milk, as it can prevent the transmission of bacteria from workers who are ill or do not practice proper personal cleanliness. Dirty hands, for instance, can contaminate milk during milking.[20]

Furthermore, based on previous research conducted in Jember Regency, results showed that there is a relationship between personal hygiene and the presence of S. aureus in cow's milk. Personal hygiene in that research included the cleanliness of hands and nails (p-value 0.003), hair cleanliness (p-value 0.011), and clothing cleanliness (p-value 0.027).[8]

Another study conducted in Jember Regency in 2023 found a relationship between the hygiene of milkers, particularly the habit of washing hands before milking, and S. aureus contamination in cow's milk, with a p-value of 0.036. However, the same study concluded that there was no relationship between clothing cleanliness and S. aureus contamination in cow's milk, with a p-value of 0.111. This could be due to other factors playing a greater role in determining the level of milk contamination.[15]

To maximize the safety of milk handling and avoid bacterial transmission from handlers to the milk, milkers need to practice personal hygiene in accordance with guidelines. Farmers should change into clean clothes before beginning the milking process. Clothing that has been exposed to cow manure or urine during barn cleaning can be a source of microorganism contamination. Milkers should also routinely trim their nails. Having short and clean nails will prevent dirt from getting trapped and accumulating under the nails, thereby reducing the risk of bacterial contamination in the milk.[8]

Milkers must always practice proper handwashing with soap before and after milking. They are also expected to correctly use head coverings as personal protective equipment to ensure their hair is completely covered. Smoking should be strictly prohibited in the milking area. Not only does smoking risk adding contaminants to the milk, but it also lowers air quality around the milking area. In addition, milkers are encouraged to practice proper sneezing and coughing etiquette—that is, always covering their mouth and nose with a tissue or the inner arm when sneezing or coughing, and washing their hands afterward. This is vital to prevent the spread of bacteria and viruses during the milk handling process.

Microorganisms that commonly contaminate milk, such as S. aureus, may originate from the surrounding environment or milk handling equipment. These microorganisms can quickly grow and multiply, which will ultimately spoil the milk and directly decrease its quality, potentially causing health issues for consumers.[18]

Moreover, unhygienic milking procedures—including those relating to the farmer, livestock, barn and the farm environment, as well as milk handling equipment—can act as means of transmitting microorganisms into the milk. Microbial contamination can cause chemical changes and shorten the milk's germicidal lifespan. In the end, the milk will also experience physical changes in color, taste, and aroma. Therefore, to maintain the safety and quality of milk, preventing microbial contamination through hygienic milking procedures is necessary.[18]

Conclusion

This study shows that the sanitation of barns, milking equipment, and the personal hygiene of milkers in the livestock farmer groups in Baturaden and Sumbang Districts mostly do not meet the standards, thereby contributing to the high contamination of Staphylococcus aureus in fresh cow's milk. There is a significant relationship between these three factors and contamination, with the personal hygiene of milkers being the most influential factor. These findings underscore the importance of improving sanitation and hygiene to ensure the quality and safety of fresh milk.

Conflict of interest

The authors declare no conflicts of interest.

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