THE INDONESIAN JOURNAL OF MEDICAL LABORATORY TECHNOLOGY
https://jurnal.stikesbanyuwangi.ac.id/index.php/IJ-CALORY
Volume 1, No. 1, January 2024 (Page. 7-13)

Total Plate Count, Most Probable Number Index, And Coliform Bacteria Of Fresh Cow Milk In Jember Traditional Market

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ARTICLE INFORMATION

Article history
Received (01st December 2023)
Revised (16th December 2023)
Accepted (12nd January 2024)

Keywords
Coliform, Most Probable Number, Raw Cow Milk, Total Plate Count

ABSTRACT

Introduction: *Jember is an area with habit of fresh cow milk consumption. It is presumed to provide the best nutritional source for individual health.*
Objectives and Methods: *this study aimed to analyze total plate count, most probable number, and coliform bacteria in fresh cow milk in Jember traditional market. An analytical descriptive research was performed on 30 samples of raw cow milk from 5 different merchants in Jember traditional market from September to October 2023.*
Results: *The results showed that 7 (23.3%) samples in this study met the TPC requirement but 23 (76.7%) samples exceeded the TPC requirement by SNI. In addition, 10 (33.3%) samples met the MPN requirements but 20 (66.7%) samples exceeded the MPN requirement by SNI.*
Conclusions: *It was concluded that most of fresh cows milk in Jember traditional market were not eligible to consume due to high TPC, MPN and coliform contamination. Common coliform bacteria found in Fresh cows milk sold in Jember traditional market were Escherichia coli, Enterobacter sp., and Klebsiella sp., respectively. Further study was needed to identify coliform species and analyze other parameters of fresh cows milk in Jember traditional market according to SNI.*

Introduction

Foodborne diseases are still a threat in global world, both in developed and developing countries, with estimated one case of gastroenteritis in every six people in the United States by the Centers for Disease Control and Prevention (CDC) (Todd, 2020). In addition, WHO estimates that approximately 500 million people had this condition every year, with more than 1 million deaths worldwide (Nurmawati et al.,2019). Data from the Directorate General of Disease Prevention and Control, Indonesian Ministry of Health shows that there are 1,076,555 cases of diarrhea in East Java Province in 2020, indicating that diarrhea is still a serious problem in Indonesia (Ministry of Health, 2021). This disease is caused by pathogenic microbes or their products that contaminate food and drinks (Haskito et al., 2019). As one of the nuttritious cattle products, milk may contribute to foodborne illnesses due to the potential for microbial development in it if the process is not carefully monitored. Advanced sterilization methods such as pasteurization is one serious attempt to overcome this (Grace et al., 2020).

Consuming fresh cow's milk remains a typical trend in society due to conventional mindset suggesting it will provide the best benefits, the sterilization process is destructive to nutritional quality, and consuming fresh milk is a wise strategy to maintain local dairy farms, and as a custom to maintaining body health (Adetunji et al., 2020). Health aspects of fresh cow milk is supported

by the presence of indigenous bacteria with probiotic potential playing a vital role in the body's defense by preventing bacterial colonization pathogens, and their promising role in the dairy industry (Nero & De Carvalho, 2019). There have been many studies that have found coliform bacteria in fresh cow milk because the stall area, cow feed and drink, instruments milking, and milking techniques do not meet hygiene standards and sanitation (Salman & Hamad, 2011). Therefore, coliform bacteria are often used as an indicator of the cleanliness of food and beverage products and other bacterial contamination due to poor sterilization (Tominaga & Ishii, 2020). Coliform bacteria are gram-negative bacilli with no ability to form spores but are able to ferment lactose (lactose fermenter) characterized by the production of gas and acid after incubation at 35^{o}C. This group of bacteria consists of the genera *Escherichia, Klebsiella, Enterobacter*, and *Citrobacter* (Sengupta & Saha, 2013).

The Indonesian government sets regulations on the quality of fresh cow milk to make it suitable for consumption. This is implemented to maintain public health of consumers of fresh cow milk. One of The provisions are regulated in SNI 3141.1:2011 which states that fresh cow milk suitable for consumption must not have a total plate number exceeding 1x10^{6} CFU/ml (Badan Standarisasi National, 2011). Many studies have shown that fresh cow milk traded in several places regions in Indonesia do not meet SNI quality requirements. Santoso et al reported that the lowest total plate number of fresh cow milk samples of 13 retailers in Semarang City amounted to 3.5x10^{6} CFU/ml, and MPN index coliforms was 40 – 2400 per ml, which exceeded the maximum limit total plate and MPN coliform regulated by SNI of 1x10^{7} CFU/ml and 2x10^{6} MPN/ml, respectively. (Santoso et al., 2012). Suhailah in his 2018 research in 2018 also found coliform bacteria in fresh cow milk samples from the farm area of Sukodono Sidoarjo, indicating that those fresh cow milk still did not meet SNI requirements (Suhailah & Santoso, 2018).

Jember is an area with some active cow farms and consumption of fresh cow milk. There have been not many studies performed on the microbiology analysis of fresh cow milk in Jember traditional market, and there has been no research on coliform bacteria of cow milk in Jember traditional market. Based on the background above, the author aimed to analyze the total plate count, most probable number index and identification of coliform bacteria in fresh cow milk in Jember traditional market.

Material and Methods

1. Design, place and time

An analytical descriptive research was performed on 30 fresh cow milks sold in 5 different merchants in Jember traditional market from September to October 2023.

2. Materials and instruments

Materials used in this study were: saline solution, Nutrient Agar (Merck), Lactose Broth (Merck), Brilliant Green Lactose Broth (Merck), Eosin Methylene Blue Agar (EMBA) (Merck), Triple Suger Iron Agar (TSIA) (Himedia), Simmon's Citrate (SC) (Himedia), Methyl Red-Voges Proskauer (Himedia), Buffered Peptone Water (BPW) (Merck), KOVAC's reagent (Himedia), KOH 40% (Arkitos Chemicals), Methyl red 1% w/v (Merck), and Alpha Naphtol 5% b/v (Merck).

Instruments used in this study were: autoclave, incubator, Laminary Air Flow, and colony counter.

3. Research Procedure (for laboratory-based research)

a. Total plate count

A total of 5 tubes and Sterile petri dishes were prepared and all the tubes were filled with 9 mL of saline solution. A total of 1 mL of sample was pipetted into the first tube, thereby making a total volume of 10 mL and a dilution of 1:10 (10^{-1}). Solution was homogenized and 1 mL of solution was aseptically removed from the tube first to the second tube to create a dilution of 1:100 (10^{-2}). This step was repeated sequentially until a dilution of 1:100,000 was created (10^{-2}). A total of 1 mL of sample in each tube was pipetted into each sterile petri dish before aseptic transfer to NA media using the pour plate method (Aria Suzanni et al., 2021).

b. Most probable number index

The MPN index was determined through three stages, namely the preliminary stage (presumptive test), confirmatory stage (confirmed test), and completed stage. In the preliminary stage, the diluted milk sample was added to Lactose Broth (LB) media (Merck, Darmstadt, Germany) in the tube and the Durham tube was inserted upside down into it. Samples were incubated for 24 hours at temperature of 37^{o}C; gas production and turbidity after the incubation period was recorded as a positive result. Positive results at stage presumptive test was followed up with a confirmatory stage where the completely diluted sample was added to Brilliant Green Lactose Broth (BGLB) media (Merck, Darmstadt, Germany) in tubes and durham tubes was reversedly put inside itt. Samples were incubated for 24 hours at temperature 37^{o}C; gas production and turbidity after the incubation period were recorded as positive results. The number of tubes that gave positive results was recorded and the total number of coliform bacteria interpreted by comparing the results obtained in the research this with the standard MPN table (Kumalasari et al., 2018).

At the completed stage, the BGLB media containing samples that showed positive results were inoculated on Eosin Methylene Blue (EMB) media (Merck,Darmstadt, Germany) and were incubated for 24 hours at 37^{o} C. The colony grown on EMB was stained with Gram stain. Findings of gram bacteria negative on microscopic observation indicated the positive presence of coliforms in the sample.

Identification of coliform bacteria in this study was carried out through biochemistry tests using several media consisting of Triple Sugar Iron Agar (TSIA) (HiMedia, Mumbai, India), Simmon's Citrate (SC) (HiMedia, Mumbai, India), Methyl Red-Voges Proskauer (MRVP) (HiMedia, Mumbai, India), Buffered Peptone Water (BPW) (Merck, Darmstadt, Germany). Identification stage began with inoculation of colonies grown on EMB media into TSIA, MRVP, SC, and BPW were continued with incubation for 24 hours at temperature 37^{o}C. After the incubation period, the results on TSIA and SC media were recorded, Methyl Red 1% w/v was added into MR media, Alpha Naphtol 5% w/v and KOH 40% w/v were added to VP medium, while Indole KOVAC reagent was added to BPW as an Indol test (Sari et al., 2019). The results of the total plate count, MPN index, and identification of coliform bacteria in this study were analyzed descriptively and presented in a table.

c. Data processing and analysis

The data of total plate count and most probable number index were analyzed descriptively and presented in a table.

Results and Discussion

Among the 30 fresh cow milk samples in this study, 16 (53.3%) samples showed high total plate counts and were reported as Too Numerous To Count (TNTC) (can be seen in Table 1).

Table 1: Total Plate Count Results

Sample	Colony at each dilution					TPC
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	%
1	TNTC	69	13	3	1	$6.9x10^{3}$
2	TNTC	TNTC	51	0	0	$5.1x10^{4}$
3	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
4	TNTC	73	11	1	0	$7.3x10^{3}$
5	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
6	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
7	TNTC	173	104	28	25	$2.9x10^{6}$
8	TNTC	TNTC	153	63	17	$7.8x10^{5}$
9	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
10	283	229	165	135	93	$1.1x10^{7}$

11	28	0	0	0	0	28
12	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
13	TNTC	TNTC	TNTC	TNTC	114	$1.1x10^7$
14	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
15	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
16	TNTC	TNTC	TNTC	TNTC	267	$2.7x10^7$
17	279	196	73	23	3	$9.2x10^4$
18	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
19	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
20	TNTC	TNTC	86	75	59	$6.7x10^6$
21	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
22	TNTC	TNTC	TNTC	TNTC	141	$1.4x10^7$
23	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
24	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
25	TNTC	TNTC	TNTC	73	10	$7.3x10^5$
26	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
27	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
28	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
29	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
30	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC

Several references state that TNTC is used to indicate bacterial contamination > 300 CFU/petri (Sutton, 2011). The MPN test results in this study showed that 9 (30%) of the 30 samples had the highest MPN index >1,100 per ml sample. However, 10 (33%) of the 30 samples had the lowest MPN Index of 0 per ml of sample, indicating the absence of coliform bacteria in these samples.

Biochemical tests using TSIA, Indol, MR, VP, SC media were only carried out on 20 (67%) samples with positive MPN results to further identify the genus of coliform bacteria that contaminated the samples. The results showed that *E. coli* was the most common bacteria found in this study, namely in 11 (36.7%) samples. This was characterized by a metallic green sheen on EMB media, yellow color on the base and slopes of TSIA, negative H2S production but positive gas production on TSIA, positive indole, negative MR, positive VP and positive results on SC media.

Enterobacter sp. was found in 5 (17%) samples, characterized by yellow color at the base and slopes of TSIA, negative H2S production but positive gas production on TSIA, positive Indole, negative MR, negative VP, and positive results on SC media. The fewest coliform bacteria found in this study were *Klebsiella sp.* namely in 4 (13.3%) samples; This was indicated by red color at the base and slope of TSIA, negative H2S production and gas production on TSIA, positive indole, negative MR and VP, and positive results on SC media.

Referring to SNI 3141.1:2011, the maximum total plate count allowed for fresh cow milk is 1x10 CFU/ml which indicated bacterial contamination in all sample in this study. Only 7 (23.3%) samples in this study met the requirements. In addition, the majority (56.7%) of the samples in this study showed Too Numerous to Count (TNTC) results on the total plate count (Table 1) which indicated poor quality of sample which was not suitable for consumption. This was in accordance with research by (Pramesthi & Hari, 2015) which reported that fresh milk at the Mulyorejo Farm in Semarang had an average total plate number of $3.15x10^6$ CFU/mL, thereby did not meet SNI quality standards.

Coliform with a maximum limit of $2x10^6$ colonies/mL analyzed using the MPN method is one of the microbiological quality standards for fresh cow's milk required by SNI 7388: 2009. This study found that only 10 (33%) samples in this study met the SNI criteria. and coliform bacteria were found in 20 (67%) samples in this study. This finding was in line with a study by (Kusumaningsih & Ariyanti, 2013) which showed that 33 out of 34 (97.06%) fresh cow milk in dairy farming centers in Cibungbulang, Bogor exceeded the maximum SNI MPN limit.

This study found the most *E. coli* contamination in fresh cow milk, namely in 11 (36.7%) samples. This finding was in accordance with research (Pradika et al., 2019) which found *E. coli* in 31.3% of fresh cow milk samples at one of the cattle farms in Banyuwangi Regency. In addition to its role as normal flora in the intestines of healthy animals, *E. coli* is the most common causative agent for foodborne illnesses. Consuming fresh milk products is an ideal condition for the transmission of this bacteria because of its heat-labile nature supported by several virulence factors such as Shiga-like toxin which triggers bloody diarrhea, the Intimin gene which mediates the attachment of host intestinal cells, and fimbriae to facilitate rapid bacterial colonization (Abebe et al., 2020).

This research found contamination with *Enterobacter sp.* in 5 (17%) sample, in accordance with research (Mokadem et al., 2020) which found Enterobacter sp. in 15% of fresh cow's milk samples, with most species Many identified are Enterobacter aerogenes. Another study by (Younis et al., 2017) in Egypt found E. aerogenes, E. agglomerans and E. cloacae among 100 fresh milk collected from cows infected with subclinical mastitis. The natural habitat of Enterobacter sp. are land and water, thereby allowing bacteria to interact with or infect each other plant. In addition, Enterobacter sp. known for its role as a flora normal intestine, although it has a great ability to cause hospital outbreaks among inpatients with circumstances immunocompromised. E. aerogenes and E. cloacae are two species of the genus Enterobacter is reported as the most common speciesfound in clinical samples (Davin-Regli et al., 2019).

Klebsiella sp. is the least common bacteria found in this study, namely only 4 (13.3%) milk samples, in accordance with the study (Yang et al., 2021) which detected Klebsiella sp in 9.78% of milk samples in Jiangsu and Shandong Provinces, China. Among the Klebsiella species, K. pneumoniae is the most common bacteria and is positively correlated with clinical mastitis of milk. Apart from K. pneumoniae, K. oxytoca is also known for Its pathogenicity causing clinical mastitis in cows is characterized with greatly reduced milk production. Although the pathogenesis is still not yet known with certainty, several virulence factors such as magA (determinant the ability of bacteria to form mucosal filaments), uge, gene (encoding capsule and antigenic wall structures) and the rmpA gene (determinant mucoid properties) are thought to play a role in bacterial pathogenicity (Massé et al., 2020).

High amounts of coliform bacteria in food or drink has the potential to cause digestive problems, namely gastroenteritis or diarrhea due to the toxins it produces (Muthaz et al., 2017) (Saputri & Efendy, 2020).

The contamination of coliform bacteria in fresh cow's milk can be caused by cleaning equipment for milking equipment, milk storage containers, the milking process, and milkmaid hands that are not clean or hygienic. The absence of a sterilization process after milking is also a factor that facilitates bacterial contamination (Asfidoajani & Sichani, 2018).

Conclusion

Based on the results of the research that has been carried out, it can be concluded that only 7 (23.3%) samples in this study met the plate number requirements total SNI, while 23 (76.7%) exceeded the maximum total plate number limit SNI. In addition, 10 (33.3%) samples met the MPN requirements while 20 (66.7%) the sample exceeds the SNI maximum MPN limit for fresh cow's milk. Can be concluded that most of the fresh cow's milk in Banyuwangi Regency is still not available safe to consume because it still exceeds the maximum ALT and MPN limits SNI is required, and because it still contains coliform bacteria The species most frequently found were E. coli and Enterobacter, respectively sp., and Klebsiella sp. Further research needs to be done for identification coliform bacteria species as well as carrying out other inspection parameters as a form of testing the quality of fresh cow's milk in Banyuwangi Regency based on SNI quality requirements.

Suggestions

The author suggests that breeders pay more attention to hygiene and animal health, livestock environment, and sterilization methods used. The public as the main consumer of fresh cow's milk needs to increase their awareness about the dangers of consuming contaminated fresh cow's milk. This cannot be separated from the government's role in socializing the quality requirements for fresh cow's milk that need to be met by farmers so that cow's milk can be produced that is suitable for consumption by the public

Acknowledgments

Authors would like to thank Universitas Dr. Soebandi for the financial and technical support during the research.

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