Risk Factors for Bacterial Contamination of Bovine Meat during Slaughter in Ten Indonesian Abattoirs

Diyantoro and Dhandy Koesoemo Wardhana

1Department of Health Science, Faculty of Vocational Studies, Universitas Airlangga, Surabaya, Indonesia
2Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

Correspondence should be addressed to Diyantoro; diyantoro_dvm@vokasi.unair.ac.id

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Provision of beef meat which does not exceed the maximum microbial contamination limit is expected to meet the requirements to obtain safe, healthy, wholesome, and halal beef. Bacterial contamination during slaughtering process is a safety problem and concern for shelf life in meat production. This study was designed to determine the value of microbial contamination and its risk factors at the stage of the slaughtering process in the abattoirs. This research was conducted by visual observation accompanied by questionnaires and laboratory examination for bacterial contamination testing. The results showed the factor that significantly affected the total plate count (TPC) was carcass cutting (mean: $0.46 \times 10^6$ CFU/g; $p = 0.035$) which was not carried out by the abattoir. The factor that had the greatest effect on the MPN of *Escherichia coli* was blood removal on the floor position (mean: $40.34 \times 10^6$ CFU/g; $p = 0.039$) while the factors that significantly affected *Staphylococcus aureus* contamination were blood removal on the floor position (mean: $52.88 \times 10^6$ CFU/g; $p = 0.025$) and carcass cutting which were not carried out by the abattoir (mean: $66.42 \times 10^6$ CFU/g; $p = 0.015$).

1. Introduction

Food of animal origin should be monitored to ensure that people can obtain consumable meat. Beef meat may include biological, physical, and chemical hazards that may occur at any point during the supply process from slaughtering to table. Pathogenic microorganisms are normally found in the digestive tract of healthy cattle. These microorganisms can also be found on the hides of live animals contaminated from feces which can then be transferred to the surface of previously sterile meat during slaughtering especially when performed on the floor with the absence of a carcass suspension system with careless evisceration that spreads intestinal content onto the meat surface. Bovine carcasses can be contaminated during the slaughter process through contact with the animal’s skin and hair, limbs, blood, stomach, gut contents, bile, and other excretions, facilities, equipment, and hands and worker’s clothes [1]. For those reasons, special attention is needed in the implementation of hygiene and sanitation during the slaughtering process. The important steps preparing for the quality and safe meat occur in abattoirs. Abattoirs are a community service unit that are intended to provide safe, healthy, and wholesome halal meat, a place for hygienic slaughter, and a place for monitoring and surveillance of animal diseases and zoonoses [2].

According to the Directorate General of Animal Husbandry and Animal Health, Ministry of Agriculture Republic Indonesia [3], there are only 25 abattoirs of around 800 abattoirs in Indonesia that have a veterinary certificate, which are official and legal benchmarks indicating hygiene-sanitation requirements as basic feasibility guarantee of food safety from animals produced by the abattoir. Article 62 of Law 18/2009 concerning animal husbandry and animal health states that the district or city government must have an abattoir that meets technical requirements. From this statement, it is clear that the law mandates regional governments to fulfill the technical requirements of abattoirs in their territory. However, in reality, the abattoir has the main function for providing consumers with halal slaughtered livestock, safe meat and maintaining the quality of produced-meat, although at present this requirement is not always fulfilled. The supply of beef meat which does not exceed the maximum microbial contamination limit is expected to meet the above requirements to provide safe, healthy, wholesome halal beef. An abattoir is not a sterile
environment and has a high risk of pathogenic microbial contamination. After the cattles are processed, the microflora found in animals begin to invade the tissue so that the meat will spoil quickly if the product is not handled correctly [4].

The previous study reported that several meat samples from city slaughterhouses in East Java, Indonesia was found to have microbial contamination including \textit{Escherichia coli} (32.5\%), \textit{Staphylococcus aureus} (20\%), and \textit{Salmonella} sp. (2.5\%) [5]. In addition to the highest prevalence rate, the mean value of \textit{Escherichia coli} contamination was higher than the maximum limit of microbial contamination based on the National Standardization Agency of Indonesia [6]. Good Slaughtering Practices (GSP) includes all practices in abattoir relating to the conditions and actions needed to ensure the safety of food at all stages in the food chain [7]. Harris and Jeff [8] stated that the implementation of GSP serves to minimize the contamination of diseases from preslaughter, handling the livestock in the lairages washing, stunning, slaughtering, and carcass washing. In addition, the GSP stages should also include the cleanliness of production facilities, water used during the process, implementation of sanitation programs, and validation processes. This study was aimed to determine the value of microbial contamination including total plate count (TPC), \textit{Staphylococcus aureus}, and \textit{Escherichia coli} and its risk factors at the stage of the slaughtering process in abattoirs.

2. Materials and Methods

2.1. Abattoirs Selection. Ten city abattoirs in East Java Province were selected in this study including Kedurus Abattoir and Pegirian Abattoir located in Surabaya City, Mojokerto City Abattoir, Pasuruan City Abattoir, Gadang Abattoir located in Malang City, Batu City Abattoir, Kediri City Abattoir, Probolinggo City Abattoir, Dimori Abattoir located in Blitar City, and Madiun City Abattoir.

2.2. Observation and Data Collection. General characteristics and slaughtering processes were observed visually from each abattoir. The slaughtering process, such as slaughtering, blood removal techniques, meat cutting, rigor mortis process, skin preparation and evisceration process, and subcutaneous fat trimming, were carefully perceived.

2.3. Meat Sample Collection. Four samples from each abattoir were collected from ten city abattoirs. The samples were collected with an attempt to minimize microbial contamination caused by environmental temperatures; hence it was done in the early morning and within 8 hours postslaughter. One hundred grams of raw beef meat (gluteus medius) samples were collected from each sample. Then ten grams of the collected meat sample were further transferred to sterile flask containing 90 ml of distilled water. Under aseptic condition, the samples were homogenized using the pestle and mortar.

2.4. Microbiological Examination

2.4.1. Total Plate Count (TPC). Total Plate Count was performed initially by homogenized 25 grams of each meat sample with 225 ml of 1% Buffered Peptone Water (BPW) (Merck 1.07228.0500) for 1-2 minutes then put it into serial dilution. Five sterile test tubes were labeled as $10^{-1}$ to $10^{-5}$ for serial dilution. One ml of diluted meat sample was mixed thoroughly with nine ml of BPW in the first test tube and labeled as $10^{-1}$. One ml solution was taken from the first test tube and transferred to the second test tube labeled as $10^{-2}$. This was continued until $10^{-5}$ dilution was obtained. Then one ml of meat samples from each dilution was inoculated on Nutrient Agar (NA) (Merck 1.05450.0500) plates, and then incubated at 37°C for 18–24 hours. After 24 hours, the observed growing colonies from the NA plates were counted for \textit{Total Plate Count} (TPC) [9].
2.4.2. Most Probable Number (MPN) Escherichia coli. The viable numbers of Escherichia coli in a sample were estimated using the MPN method. One ml sample was added into 9 ml BPW media then a serial dilution of three test tubes were set as 10⁻¹ to 10⁻³. One ml of each diluted samples were transferred into five tubes containing brilliant green bile broth (BGBB) media (Merck 1.05454.0500) with inserted Durham tube and incubated at 45.5°C for 24−48 hours. Escherichia coli were observed in those tubes which produced gas. The confirmation test was performed by inoculating 1 loop of positive Escherichia coli-containing broth into an Eosin Methylene Blue Agar (EMBA) (Merck 1.01347.0500). Green metallic appearance will be observed for sample containing Escherichia coli. These samples then further proceeded for indol testing and transferred into tryptone water media (Merck 1.10859.0500). The MPN value was calculated based on the number of tubes with positive Escherichia coli broth dilution using McGrady’s table [10].

2.4.3. Detection of Staphylococcus aureus. Staphylococcus aureus was detected with inoculation of 0.1 ml from the first dilution (10⁻¹) into Mannitol Salt Agar (MSA) (Merck 1.05404.0500) after 24 hours at 37°C. Staphylococcus aureus gave yellow colony appearance on MSA while other species of Staphylococcus showed red colonies [11].

2.5. Data Analysis. Data from visual observation in the field and laboratory tests is analyzed by one-way analysis of variance (ANOVA) using SPSS [12] statistical software (Ver.16.0 for windows, SPSS Inc, Chicago, IL, USA) to determine the significance (p-value) of the mean values of each Total Plate Count, Staphylococcus aureus, and Escherichia coli tests between categories of each parameter was tested. In addition, a regression analysis test was also conducted to determine the percentage of positive sample and Odd Ratio (OR) value of each parameter for determining the risk factors or exposure associations of microbial contamination.

3. Results and Discussion

The general information of the ten selected abattoirs in Table 1 showed that most abattoirs were owned by service technical implementation units. Meanwhile, based on the

### Table 2: Total plate count.

| Slaughtering process stage | Category | % pos. | OR | p value | Mean (10⁶ CFU/g) | CI | p value |
|---------------------------|----------|-------|----|---------|-----------------|----|---------|
| Blood removal techniques  | Hanging position | 6.3 (1/16) | 1.5 | 0.769 | 0.16 | (0.03)–0.36 | 0.206 |
|                          | On the floor position | 4.2 (1/24) | Ref. | 0.33 | 0.15–0.50 | | |
|                          | Not carried out | 8.3 (1/12) | 0.4 | 0.538 | 0.46 | 0.13–0.79 | 0.035* |
|                          | Carried out by abattoir | 3.6 (1/28) | Ref. | 0.18 | 0.06–0.29 | | |
| Carcass cutting          | Cut into quarters | 12.5 (1/8) | 7.0 | 0.999 | 0.38 | (0.14)–0.89 | 0.410 |
|                          | Cut into halves | 4.2 (1/24) | 2.3 | 0.999 | 0.27 | 0.12–0.43 | | |
|                          | Cut into other sizes | 0.0 (0/8) | Ref. | 0.11 | 0.01–0.22 | | |
| Rigor mortis process     | Yes | 25.0 (1/4) | 11.7 | 0.110 | 0.36 | (0.73)–1.44 | 0.624 |
|                          | No | 2.8 (1/36) | Ref. | 0.25 | 0.13–0.37 | | |
| Skin separation and evisceration process | Hanging position | 8.3 (1/12) | 2.5 | 0.538 | 0.27 | (0.06)–0.59 | 0.954 |
|                          | On the floor position | 3.6 (1/28) | Ref. | 0.26 | 0.13–0.39 | | |
| Subcutaneous fat trimming | Yes | 8.3 (2/24) | 1.5 | 0.999 | 0.34 | 0.14–0.54 | 0.132 |
|                          | No | 0.0 (0/16) | Ref. | 0.15 | 0.05–0.25 | | |

*Indicates a significant difference of p < 0.05.

### Table 3: MPN Escherichia coli.

| Slaughtering process stage | Category | % pos. | OR | p value | Mean (10⁶ CFU/g) | CI | p value |
|---------------------------|----------|-------|----|---------|-----------------|----|---------|
| Blood removal techniques  | On the floor position | 45.8 (11/24) | 0.2 | 0.039* | 40.34 | 7.56–73.13 | 0.097 |
|                          | Hanging position | 12.5 (2/16) | Ref. | 7.05 | 3.43–10.67 | | |
|                          | Not carried out | 50.0 (6/12) | 0.3 | 0.129 | 32.25 | (9.95–74.25) | 0.737 |
|                          | Carried out by abattoir | 25.0 (7/28) | Ref. | 24.83 | 1.09–48.57 | | |
| Carcass cutting          | Cut into quarters | 50.0 (4/8) | 3.5 | 0.277 | 41.73 | (25.82–109.27) | 0.534 |
|                          | Cut into halves | 33.3 (8/24) | 7.0 | 0.129 | 28.78 | 1.09–54.48 | | |
|                          | Cut into other sizes | 12.5 (1/8) | Ref. | 7.05 | 1.12–46.86 | | |
| Rigor mortis process     | Yes | 50.0 (2/4) | 2.3 | 0.440 | 14.55 | (1.49)–30.59 | 0.667 |
|                          | No | 30.6 (11/36) | Ref. | 28.41 | 6.34–50.48 | | |
| Skin separation and evisceration process | On the floor position | 39.3 (11/28) | 0.3 | 0.175 | 35.42 | 7.24–63.62 | 0.194 |
|                          | Hanging position | 16.7 (2/12) | Ref. | 7.42 | 2.56–12.27 | | |
| Subcutaneous fat trimming | Yes | 33.3 (8/24) | 0.9 | 0.890 | 20.28 | 0.14–40.45 | 0.406 |
|                          | No | 31.3 (5/16) | Ref. | 37.15 | (5.25)–79.55 | | |

*Indicates a significant difference of p < 0.05.
Table 4: *Staphylococcus aureus*.

| Slaughtering process stage               | Category                  | % pos. | OR   | p value | Mean (CFU/g) | CI          | p value |
|------------------------------------------|---------------------------|--------|------|---------|--------------|-------------|---------|
| Blood removal techniques                 | On the floor position     | 25.0 (6/24) | 0.2  | 0.156  | 52.88        | 31.54–74.21 | 0.025*  |
|                                          | Hanging position          | 6.3 (1/16) | Ref. |         | 19.56        | 2.49–36.63  |         |
|                                          | Not carried out           | 33.3 (4/12) | 0.24 | 0.099  | 66.42        | 30.00–102.83| 0.015*  |
|                                          | Carried out by abattoir   | 10.7 (3/28) | Ref. |         | 28.04        | 13.83–42.24 |         |
| Carcass cutting                          | Cut into quarters         | 62.5 (5/8) | 1.0  | 1.000  | 55.75        | 3.04–108.46 | 0.532   |
|                                          | Cut into other sizes      | 25.0 (2/8) | 0.4  | 0.408  | 31.00        | (9.05)–71.05|         |
|                                          | Cut into halves           | 0.0 (0/24) | Ref. |         | 37.00        | 19.83–54.17 |         |
|                                          | No                        | 19.4 (7/36) | 0.0  | 0.999  | 41.58        | 25.42–57.75 | 0.415   |
| Rigor mortis process                     | Yes                       | 0.0 (0/4) | Ref. |         | 21.25        | (32.01)–74.51|         |
|                                          | No                        | 12.5 (2/16) | Ref. |         | 29.50        | 8.72–50.28  |         |

Table 3 showed that the only factor having a statistically significant effect on MPN of *Escherichia coli* was blood removal performed on the floor with a positive percentage of 45.8%, the mean value of 40.34×10⁶ CFU/g, and p-value of 0.039.

There were two stages of the slaughtering process that had a significant effect on the value of *Staphylococcus aureus* contamination including blood removal performed on the floor (p = 0.025) and carcass cutting which was not carried out by abattoir (p = 0.015). The percentage of positive samples of *Staphylococcus aureus* contamination in the blood removal stage was 25% with the mean value of 52.88×10⁶ CFU/g, while in the carcass cutting stage was 33.3% with the mean value of 66.42×10⁶ CFU/g (Table 4).

Blood removal performed on the floor is very unhygienic. The process should take place on a clean stainless-steel table which should be cleaned frequently. One of the main sources of contamination during bleeding is knife. The knife should be changed after operation and returned to a sterilizer [16]. Bleeding and skinning of neck, cheeks, shoulder, and legs were done on the floor and contamination from the hide of one animal to others was transmissible. In the cattle slaughter line, all the slaughtering processes were performed on a production line with vertical rail dressing and automatic hide pullers. Hygienic condition of bleeding in cattle slaughter line was better when the animals hoisted by one leg and bleeding continues until the blood flow is negligible. In general, contamination of carcasses is reduced by using automatic hide removal because there is less handling of the carcass and less use of knives. Vertical rail dressing improves hygienic practice by reducing carcass contact with operators, equipment, and other carcasses [17].

The cutting of carcasses, involves the use of utensils, equipment and knives and may allow for the transfer of more microorganism to beef tissues. Furthermore, workers’ hands, clothes and their instruments may spread contamination onto the surface of beef carcasses [18]. In most developing countries, the absence or poor hygienic practices in slaughtering, dressing and evisceration has been found to be one of the major causes of high surface contamination of beef carcasses by pathogenic and nonpathogenic microorganism [19].

One of the main sources of higher values of staphylococcal counts on the surface of examined carcasses are abattoir workers, as their hands were found to be highly contaminated; this is in accordance with the reports of Schlegelova et al. [20]. Additionally, Dickson and Anderson [21] and Sokari and Anozie [22] mentioned that contaminated skin, faeces, the contents of digestive organs, butchers’ knives, hands, clothes, and contaminated water are the main sources of contamination with Staphylococcal spp. during meat processing.
The surface contamination of beef carcasses with coliforms could be attributed to contamination from their intestine; however, hides and hooves contain a large number of such organisms from soil, manure, and feed and may be transferred to the carcass during dressing. Besides, the contaminated water, utensils, and equipment used in carcass slaughtering, dressing, and evisceration, these results support the views previously reported of Guthrie [23]; Gracey and Collins [18] and Marriot [24].

4. Conclusions

There were several stages of the slaughtering process that had significant effects on microbial contamination. The only factor that had a significant effect on the TPC value was carcass cutting which was not carried out by an abattoir, while with regard to the MPN value, the only significant value related to blood removal performed on the floor. There were two stages of the slaughtering process that had a significant effect on the value of *Staphylococcus aureus* contamination and again these included blood removal performed on the floor and carcass cutting which was not carried out by the abattoir.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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