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Original Research Article

Bacteria contamination of surfaces and facilities at the ultra-modern abattoir Ilorin, North Central, Nigeria

[1]*Odetokun, I.A., [1]Atane, A., [1]Mohammed, K.M., [2]Alhaji, N.B., [1]Ghali-Mohammed, I. & [1]Elelu, N.

[1]Department of Veterinary Public Health and Preventive Medicine, University of Ilorin, Ilorin, [2]Public Health and Epidemiology Department, Niger State Ministry of Livestock and Fisheries, Bosso, Minna, Nigeria.*

*Corresponding author: odetokun.ia@unilorin.edu.ng, 08030886018

ABSTRACT

Abattoir plays a major role in the processing of meat consumed by the public and its involvement in public health safety cannot be overemphasized. This study was conducted to assess the bacterial load on beef carcasses and the hygiene status of the slaughterhouse environment and facilities at the ultramodern abattoir in Akerebiata, Ilorin, Nigeria. Assessment of microbial load was carried out using Standard Plate Count (SPC) and Total Coliform Count (TCC) on environmental swabs and meat contact surfaces (n = 231), water (n = 16) and red meat (n = 14). Significant differences and association between samples were determined using Tukey's Multiple Comparison Test and Carl Pearson Correlation Matrix, respectively, on GraphPad Prism with $p < 0.05$ considered as significant. Generally, the SPC and TCC for all samples exceeded the acceptable limits for meat and potable water set by WHO, CDC, and EU regulations. The floor had the highest mean count for SPC (8.66 ± 8.24 log cfu/cm^2) and TCC (6.02 ± 5.85 log cfu/cm^2). Water sourced from the borehole had no significant count for coliform as opposed to the water from the well (4.43 ± 4.32 log cfu/ml). There was a significant association between the contamination levels of butchers' hands and processed meat ($p < 0.05$). There is high level of bacterial contamination as indicated by the findings of this present study. This study further reiterates the public health importance of good management and hygiene practices in the meat processing chain for quality and consumer safety.

Keywords: Abattoir, bacteria contamination, bacterial count, Ilorin, meat processing.

INTRODUCTION

An abattoir also known as a slaughter house, is a licensed facility where animals are slaughtered and wholesomely processed into meat products for human consumption (FAO, 2021). The operation of abattoirs could be beneficial to humans by providing meat that are fit and wholesome for human consumption and other useful by-products, and could still be of health hazards arising for meat contamination and the uncontrolled release of wastes and effluents (FAO, 2021). Edible meat from cattle slaughtered in various abattoirs remains the major meat consumed and a source of protein for the Nigerian population (Ademola, 2010). Abattoirs, where available in most places in Nigeria, operate under sub-standard conditions. Reports have shown that as a result of water scarcity in some abattoir premises, butchers clean their dressed carcasses and tripe in nearby streams contributing to human and animal fecal contamination (Arnisalo *et al*., 2006).

Foodborne diseases are considered to occur frequently and are usually associated with developing countries due to improper food handling, unhygienic practices, inadequate food safety legislation, weak regulatory systems, lack of financial resources for safety equipment and lack of awareness and/or training for butchers and other food handlers (Goja *et al*., 2013; Haileselassie *et al*., 2013). The US Centers for Disease Control and Prevention (CDC) also reported annual outbreaks of foodborne diseases from food of animal origin estimated at 76 million infections, 325,000 hospitalizations and 5,000 deaths each year in Nigeria (Aluko *et al*., 2014). The increase in the prevalence of communicable and zoonotic diseases such as tuberculosis, cysticercosis and trichinosis in communities is a further sign of the importance of abattoirs as disease surveillance points (Alton et al., 2015).

Abattoir operations generate characteristic highly organic wastes with relatively high levels of solid, liquid and fat

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(Adeyemo, 2002). Condemned meat, bones, undigested horns, feathers and aborted fetuses are among the solid waste. Usually, liquid waste is composed of dissolved solids, blood, urine, water and the contents of the gut (Eze & Eze, 2018). Animal food is always microbiologically contaminated by internal organisms naturally or externally from the surroundings through processing operations (Adegunloye, 2013). Environmental pollution as well as other health hazards endangering animal and human populations can be monitored through proper inspection processes. However, meat hygiene services function in a way that satisfies consumers while safeguarding public health and animal hygiene (Kebede, 2010; Mummed & Webb, 2015).

Public health concerns are important when linked to products of animal origin as this could be a potential source of zoonotic disease transmission (Odetokun *et al*., 2018; Odetokun *et al*., 2020). Global meat production nearly doubled between 1980 and 2004, and this trend is set to continue in the future between 2000 and 2050 (Steinfeld *et al*., 2006). The quality of meat must be ensured as an acceptable standard for the health protection of consumers. Diseases resulting from the consumption of contaminated animal products from the abattoir are not uncommon and may be associated with high levels of pathogenic bacterial contamination in processed meat as well as the consumption of improperly cooked meat (Steinfeld *et al*., 2006).

With the enumerated public health challenges associated with meat processing in the abattoir set-up, this study was embarked upon to determine the level of bacterial contamination of surfaces and facilities at the ultra-modern abattoir in Akerebiata, Ilorin, North Central Nigeria. Regarding the study location, this research will help ascertain the level of microbial load in the abattoir environment to confirm whether the practices of the meat processing plant are hygienic for the processing of meat to ensure safety of consumers' health.

MATERIALS AND METHODS

STUDY AREA

The study was conducted in Kwara State at the ultra-modern abattoir, Ilorin. The ultra-modern abattoir was purposefully selected because there is a significant number of animals (approximately 120 cattle/day) presented for slaughter daily coupled with various workers involved in the processing of meat and animal by-products. More so, the ultra-modern abattoir was recently put into use after relocating slaughterhouse workers from the Ipata abattoir.

SAMPLE COLLECTION

Sample collection was carried out weekly for a period of 12 months (November 2019 to January 2020). A total of 261 samples were collected randomly from selected slaughter units in the abattoir during active slaughter operations. The samples included water (n=16), meat (n=14), and surface swabs (n=231); butchers' knives (n=50), clothes (n=50), palmar surface of hands (n=9), foot wear (n=50), table surfaces (n=5), floor (n=31) and wall (n=36). The surface swabs were collected using sterile swab sticks. Each sterile swab stick was dipped in sterile normal saline and then swabbed on the respective surfaces at an approximate area of $1cm^2$ as described in an earlier study (Adetunji & Isola, 2011). The swabbed portion of the stick was then cut into a universal bottle containing sterile normal saline. Similarly, meat samples (5g each) were put into universal bottles containing sterile normal saline. The water samples were aseptically collected directly from the running taps of the borehole and well water fetchers into empty sterile universal bottles. All samples were transported to the laboratory in a cooler box stocked with ice packs and processed within 3-8 hours after collection (Jaja *et al*., 2018) at the Food Safety Laboratory, Department of Veterinary Public Health and Preventive Medicine, University of Ilorin, Ilorin, Kwara State.

ETHICAL CONSIDERATION

The Ethical Review Committee of the Faculty of Veterinary Medicine, University of Ilorin, approved and endorsed the research study with approval reference number: UREC/FVM/2021/03.

LABORATORY ANALYSIS

SERIAL DILUTION

In the laboratory, a 10-fold serial dilution was performed for all samples for the SPC and TCC using sterile Normal saline (Oxoid®, UK) and MacConkey broth (Oxoid®, UK) as diluents, respectively. Nine milliliters of diluents were obtained aseptically in each of the respective test tubes using sterile pipettes. One milliliter of the sample solution from the universal bottles was then dispensed into the first test tube containing 9 ml of the diluent (10^{-1}) and diluted serially until the last test tube (10^{-7}) for SPC and (10^{-4}) for TCC. These procedures were carried out as previously described (Mhone *et al*., 2011; Odetokun *et al*., 2021).

INOCULATION OF THE SAMPLE USING SPREAD PLATE METHOD (SPM)

The plate count agar (PCA;Oxoid®, UK) and MacConkey agar (MCA;Oxoid®, UK) were used to determine the SPC and the TCC. All media used in this study were prepared by strictly adhering to the instructions of the manufacturer. All prepared media on petri dishes were hot oven dried at 40-45°C and an inoculum of 0.1 ml of the dilution was applied on the surface of each agar plate, spread quickly but gently over the entire agar surface using a sterile bent rod spreader. Replicate plating was performed for each sample on the PCA and MCA plates to have quadruplicates of each inoculated

sample plates. The 10^{-6} and 10^{-7} dilutions were used for agar inoculations for the SPC while the 10^{-3} and 10^{-4} dilutions were used for agar inoculations for TCC and subsequently incubated at 30°C for 24-48 hours.

COLONY COUNTING AND CALCULATIONS OF COLONY-FORMING UNITS (CFU)

Following incubation, each cultured plate was obtained and counted to determine the number of viable Colony Forming Units (cfu). This was done using an electronic colony counter. Calculation of cfu per milliliter, cfu per gram, and cfu per cm^2 area of samples was estimated by the formula below as described in the STARLIMS system (Public Health England, 2017).

$$\text{Count} = C \div \{V(n_1 + 0.1n_2)d\} \times n_3$$

Where:

C is the sum of colonies on all plates counted,

V is the volume applied to each plate,

n_1 *is the number of plates counted at the first dilution,*

n_2 *is the number of plates counted at the second dilution,*

n_3 *is the original volume of neat suspension, and*

d is the dilution from which the first count was obtained.

(If the swab was from a measured area, the count can be divided by the area swabbed in cm^2*).*

The log_{10} *cfu/ml was estimated for each sample.*

STATISTICAL ANALYSIS

The colony counts obtained for the surface swabs, meat, and water samples were summarized using Microsoft ® Excel 2019. GraphPad prism® version 8 was used for quantitative analysis of variance (ANOVA) among the sample types. The Tukey's Multiple Comparison Test was employed to determine significant differences between the mean ± standard deviation (S.D) of each sample with every other sample. Also, the Carl Pearson Correlation matrix was used to determine the significant association between each sample. All analyses were carried out at 5% level of error.

RESULTS

In total, 261 samples; swabs from environmental samples (n=231) which comprised of those of hands and foot wears of butchers, floor and walls of slaughter halls, table surfaces and cutting tools (knives), water and meat samples (n=30) were processed to quantitatively assess the microbial load. The results of the samples for both SPC and TCC were expressed as Log_{10} Mean ± Standard Deviation (S.D) due to the high variability in the number of bacteria recorded.

STANDARD PLATE COUNT

Table I shows the results of the SPC for all samples collected from the ultra-modern abattoir, Ilorin. The swab sample with the highest count for SPC was the floor (8.66 ± 8.24 log_{10} cfu/cm^2), while hands had the lowest count (8.24 ± 7.98 log_{10} cfu/cm^2). The well water had a higher count (7.54 ± 7.55 log_{10} cfu/ml) than the borehole water (7.47 ± 7.58 log_{10} cfu/ml). The difference between the floor and all other samples was statistically significant ($p < 0.05$). The hands, meat, well water and borehole water samples have no significant differences ($p > 0.05$). There were also no significant differences between the wall and knives swabs ($p = 0.2513$) and the wall and footwear swabs ($p = 0.9981$).

Table 1: Standard Plate Counts (log_{10} cfu) for all samples collected from the ultra-modern abattoir, Ilorin

S/N	Samples	Number of samples	Standard Plate Count (SPC) (log_{10} cfu)
1	Knives	50	8.42 ± 8.07^{c}
2	Clothes	50	8.36 ± 8.07^{d}
3	Foot wears	50	8.56 ± 8.20^{b}
4	Hands	9	8.24 ± 7.98^{d}
5	Floor	31	8.66 ± 8.24^{a}
6	Tables	5	8.41 ± 7.83
7	Wall	36	8.53 ± 8.10^{bc}
8	Meat	14	7.65 ± 7.30^{d}
9	Well Water	8	7.54 ± 7.55^{d}
10	Borehole Water	8	7.47 ± 7.58^{d}

a, b, c - different letters indicate significant differences ($p < 0.05$) in mean ± S.D between samples.

Table II shows that a significant association exists between bacterial counts in the hands and meat samples ($p = 0.039$), and between bacteria count in the hands and clothes samples ($p = 0.046$).

TOTAL COLIFORM COUNT

The swab sample obtained from the floor had the highest for the TCC (6.02 ± 5.85 log_{10} cfu/cm^2), while the table surface had the lowest counts (4.76 ± 4.77 log_{10} cfu/cm^2) as shown in Table III). The Borehole water had no significant count for coliforms. The TCC found for the meat, clothes, tables, and well water samples were not significantly different among each other ($p > 0.05$) contrary to results obtained for the floor, hands, footwear and knives samples.

DISCUSSION

We found that the microbial loads (standard plate and total coliform counts) of the samples analyzed were high, especially on the floor. The association between the microbial load on the hands to the meat and cloth samples was significantly positive. Microbial loads recorded in this study were higher than the limits (< 5 logcfu/g) stipulated in reports by international standard organizations – FAO/WHO, Codex Alimentarius Commission: Report No.: CX/NEA 03/16 and Report No.: 5996 and European Commission Report No. 2073/2005. The level of contamination observed on the floor and wall samples is possibly due to the fact that these sites can be easily contaminated by microorganisms introduced by the animals along with hides and feces, as well as blood droppings and ruptured viscera. Bacteria can grow

Table II. Carl Pearson Correlation matrix showing the association between the number of bacteria in each sample (swabs, meat, and water) and that obtained in all other samples.

Samples	Knives	Clothes	Foot wears	Hands	Floor	Tables	Wall	Meat	Well water	Borehole water
Knives		0.5339	-0.064	-0. 064	-0.1170	-0.525	0.1663	0.5354	-0.3688	0.4676
Clothes			-0.1138	0.6759*	-0.0117	-0.9337	0.1083	0.4518	-0.4473	0.4428
Foot wears				0.6182	0.3153	0.428	0.0721	-0.4362	-0.0059	-0.152
Hands								0.6912**		
Floor							-0.3118	-0.1703	-0.5205	-0.9134
Tables										
Wall									-0.0088	0.3115
Meat										
Well water										
Borehole water										

* - significant: p = 0.046, ** - significant: p = 0.039

and multiply in the ridged and irregular surfaces, cracks and crevices on the floors and walls where meat particles and

Table III. Total Coliform Count (log_{10} cfu) for all samples collected from the abattoir.

S/N	Samples	Number of samples	Total Coliform Count (TCC) (log_{10} cfu)
1	Knives	50	5.51 ± 5.55^{b}
2	Clothes	50	5.23 ± 5.44^{c}
3	Foot wears	50	5.82 ± 5.84^{ab}
4	Hands	9	5.83 ± 5.60^{a}
5	Floor	31	6.02 ± 5.85^{a}
6	Tables	5	4.76 ± 4.77^{c}
7	Wall	36	5.85 ± 5.67^{a}
8	Meat	14	5.13 ± 5.12^{c}
9	Well Water	8	4.43 ± 4.32^{c}
10	Borehole Water	8	0.00 ± 0.00

a, b, c - different letters indicate significant differences ($p<0.05$) in mean ± S.D between samples.

moisture accumulate. The SPC of the floor surface as shown from the result is at par with the findings of Adetunji & Awosanya (2011) and Adeyemi *et al*. (2018) who recorded greater than 7.0 log cfu/cm^2 for the floor slaughter slabs elsewhere in the country. The mean count of butchers' footwear differed slightly from the floor, which is possible since the foot wears are constantly in contact with the slaughter hall floor.

Similarly, the microbial load result of the butchers' clothes for SPC is in agreement with research carried out by Kyayesimira *et al*. (2020), who worked on hygiene practices in small and medium slaughterhouses in Uganda

In a previous study, a few slaughterhouse workers (32.6%) reported regular use of personal protective equipment during slaughter operations (Odetokun *et al*., 2020). This poor personal hygiene shown by the workers could facilitate microbial contamination which could easily spread to meat and other surfaces in the abattoir. The hands of butchers can serve as an important source of carcass contamination. The high mean SPC of 8.24 log cfu/cm^2 obtained from the butchers' hands was higher than and comparable to the observations from Ethiopian, Nigerian, Sudan, and Ugandan abattoirs and meat shops (Gurmu & Gebretinsae, 2013; Ayalew *et al*., 2015; Elzean *et al*., 2017; Kyayesimira *et al*., 2020; Uzoigwe *et al*., 2021). The significantly positive correlation observed between bacteria load on the butchers' hands and the meat samples are expected. Since Meat processing procedures such as flaying, evisceration, carcass splitting and cutting were performed manually by the workers and hence very prone to contamination, this should be considered an important critical pointwhere adequate control measures should be implemented.

The reason for the high bacterial count on table surfaces may be due to their repeated use and presence of dried blood drips and meat fragments which could have served as an ideal medium for the growth of micro-organisms, contributing to the increase in microbial load, as was also observed by Bhandare *et al.* (2007). Adetunji & Isola (2011) in a survey of meat tables showed higher counts for enterobacteriaceae and other contaminating bacteria whose load on meat tables increased after use and meat sales. Elsewhere in Nigeria, high microbial loads were recorded on meat tables in cattle and goat abattoirs (Adetunji & Awosanya, 2011; Adetunji & Odetokun, 2011; Chikanka & Ogbonna, 2019). In Central Ethiopia, a total mean aerobic count of 6.58 log_{10} cfu/cm^2 from cutting tables was obtained (Bersisa *et al*., 2019). This is also similar to the findings of Odetokun *et al*. (2021) who reported high microbial loads on retail meat tables across the Ilorin metropolis. Therefore, this study emphasized the institution of hygiene of meat tables at the slaughterhouse.

The TCC and SPC for meat samples were 5.13 log cfu/g and 7.65 log cfu/g respectively, and are comparable to the meat microbial count of 6.9 log cfu/g and 7.8 log cfu/g previously reported from an old slaughterhouse in Ilorin (Adeyemi *et al*., 2018). The counts also surpassed the stipulated EU Regulation 1441/2007 of < 5 log cfu/g for meat microbiological Standards SPC and TCC. These counts on the meat samples indicate a high risk to consumers (Adeyemi

et al., 2018) as it has been revealed that these microbial loads are also observed at retail meat market levels across the city (Odetokun *et al*., 2021). This indicates that once the meat is contaminated at the slaughter level, there will possible contamination of other points in the meat processing chain.

Water is used in the abattoirs at different points during meat processing. As a result, the role of abattoir water in determining the microbial load of the carcass produced is important as evidenced in this study with no significant difference found between the mean bacteria count of the water and the meat samples. The mean values of TCC and SPC in the water samples from the abattoir were 4.43 $\log_{10}$ cfu/ml and 7.50 $\log_{10}$ cfu/ml respectively. These counts are comparable to the findings of Adeyemo (2002) who reported mean coliform and total bacterial counts of 4.3 $\log_{10}$ cfu/ml and 5.18 $\log_{10}$ cfu/ml in water samples respectively from an abattoir in Ibadan. However, the high SPC of abattoir well water in this study suggests contamination during the process of obtaining water from the well. Workers were observed using untidy fetchers to draw water from the well, and animals bound for slaughter were casted and dragged along the path where the well was located. It was also discovered that some workers washed carcasses close to the well. The borehole water was bacteriologically preferable to the well water because it had no detectable coliform count as opposed to the well water that had 4.43 log cfu/ml. The high bacteria load and presence of coliforms in the water exceeded the appropriate guideline values of less than 500 cfu/ml for heterotrophic plate count of potable water and the zero detectable coliform per milliliter of water (WHO, 1997; CDC, 2015).

CONCLUSION

The level of bacterial contamination at the ultra-modern abattoir in Akerebiata, Ilorin exceeded the recommended limit set by WHO, CDC and the EU regulatory standards for foodstuff (ER 1441/2007). High bacteria count of the SPC and TCC can be attributed to the lack of good hygienic practices (GHP) and good management practices (GMP) in the abattoir because the entire stages of meat processing were carried out on the floor. Abattoir workers must be trained in GMP and GHP to improve the quality and hygiene of processed meat for public health safety and satisfaction. One key responsibility of the government is to establish and provide the appropriate hygiene and environmental legislative frameworks for abattoirs and the meat sector as a whole. These must be accompanied by regulatory systems (directives) issued by the governments to implement and strictly enforce the laws.

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