Microbial Quality of Fresh Beef from Different Slaughter Facilities in Oyo State, Nigeria

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Abstract
This study was done to assess the microbial quality of fresh beef samples, water and contact surfaces from selected slaughter facilities in Oyo state, Nigeria. In a completely randomized design, a total of 127 samples were collected from three different slaughter facilities. They were analyzed for microbial load using standard procedures. Parameters measured were Total Viable Count (TVC), Total Coliform Count (TCC), Total Fungal Count (TFC), Total *Escherichia coli* Count (TEC), Total Staphylococcus Count (TSC) and Total Salmonella Count (TSLC). The results showed no significant differences ($P<0.05$) between the mean TVC and TFC of the fresh meat samples collected from the slaughter facilities. However, significant differences existed ($P<0.05$) between the means of the TCC ($\log_{10}$CFU/g) of the beef. The values were 2.30, 1.82 and 1.91 for Akinyele central abattoir, Kara Sawmill slaughter slab and Atenda slaughter slab respectively. The TVC, TFC, TCC values recorded for this study were below the standard threshold levels. The presence of *Staphylococcus* spp, *Escherichia coli* and *Salmonella* spp on contact surfaces indicates that the hygienic practices of butchers and general sanitary conditions of the slaughter house facilities are poor, therefore stricter hygiene practices are recommended in Nigerian slaughter houses and abattoirs to safeguard public health.

Keywords: Beef; Contact surfaces; Hygiene practices; Microbial load; Slaughter facilities

Qualité microbienne de la viande de bœuf fraîche provenant de différents abattoirs de l'État d'Oyo, au Nigeria

Résumé
Cette étude a été réalisée pour évaluer la qualité microbienne d'échantillons de bœuf frais, d'eau et de surfaces de contact provenant d'installations d'abattage sélectionnées dans l'état d'Oyo, au Nigeria. Dans une conception complètement randomisée, un total de 127 échantillons a été collecté dans trois installations d'abattage différentes. Ils ont été analysés pour la charge microbienne en utilisant des procédures standard. Les paramètres mesurés étaient le nombre total de viables (TVC), le nombre total de coliformes (TCC), le nombre total de champignons (TFC), le nombre total d'Escherichia coli (TEC), le nombre total de staphylocoques (TSC) et le nombre total de salmonelles (TSLC). Les résultats ont montré qu'il n'y avait pas de différences significatives (P<0,05) entre les moyennes du TVC et du TFC des échantillons de viande fraîche collectés dans les abattoirs. Cependant, des différences significatives existaient (P<0,05) entre les moyennes du TCC (log10CFU/g) de la viande bovine. Les valeurs étaient respectivement de 2,30,

1,82 et 1,91 pour l'abattoir central d'Akinyele, la dalle d'abattage de Kara Sawmill et la dalle d'abattage d'Atenda. Les valeurs TVC, TFC, TCC enregistrées pour cette étude étaient inférieures aux seuils standard. La présence de *Staphylococcusspp, Escherichia* coli et Salmonellaspp sur les surfaces de contact indique que les pratiques hygiéniques des bouchers et les conditions sanitaires générales des installations de l'abattoir sont médiocres, par conséquent des pratiques d'hygiène plus strictes sont recommandées dans les abattoirs et les abattoirs nigérians pour sauvegarder la santé publique.

Mots clés: Bœuf, Surfaces de contact ; Pratiques d'hygiène ; Charge microbienne ; Installations d'abattage.

Introduction

The demand for meat is increasing so also its concerns regarding the quality, freshness and wholesomeness (Selvan *et al.*, 2007). There have been reports on outbreaks of food borne illnesses associated with the consumption of meat (Lunden *et al.*, 2003; Prakash *et al.*, 2005; Bhandare *et al.,* 2007). The muscle tissue of healthy living animal is usually free from micro-organisms before slaughter, however, during the slaughtering and at different stages of meat processing after slaughtering, different microbes get introduced to the meat and these microbes tend to contaminate the meat (Ebel *et al.,* 2004; Sumner *et al.,* 2003). The meat is potentially subjected to contamination from various sources during the slaughtering of animals, handling and during its sale. The abattoir environment and slaughtering processes play a vital role in the whole-someness and safety of meat. The production and distribution of wholesome beef in different production outlets such as abattoir and slaughter slabs in Nigeria is still a big challenge. The absence of well-equipped abattoir or slaughter house facilities, non-compliance with food safety standards, lack of well trained personnel and the presence of unhygienic meat handling, personnel and others have been the major limitations for the production of wholesome meat in Nigeria.

The poor knowledge of sanitary practices of the butchers, sales men, operators and patrons of abattoirs in developing countries have increased consumer vulnerability to micro-bial infections through meat (Elmossalami, 2003). Dirty environment and unhygienic food handling influence wide spread of bacterial food poisoning (Tutenel *et al.,* 2003; Burgess *et al.,* 2005). The cont-aminating organisms are derived mainly from the hide of the animal, the faeces, the place of slaughter, the environment of the slaughter house, vehicle used for the transport of the meat from the slaughter house to the retail outlet, the floor of the retail outlet and the air in the outlet which all act as the external sources for the contamination of the meat (Cooper, 1999; Sofos *et al*., 1999; Sudhakar *et al.,* 2009).

Microorganisms of relevance with regard to meat include moulds, bacteria and viruses. Within these groups, bacteria play a highly significant role. The most frequently identified bacterial pathogens associated with consumption of beef products are *Staphylococcus, Salmonella, E. Coli, Listeria* and *Clostridium* (Nouichi and Hamdi 2009). The microbiological quality of meat and meat products is very important due to its significance to public health (Bhandare *et al.,* 2007). It is therefore imperative that microbial contamination of meat and meat products do not exceed the threshold levels safe for human health (5 Log_{10}cfu/g or $1x10^6$cfu/g). Furthermore, microbial loads

exceeding this threshold levels could adversely affect the shelf life of meat and meat products and render it unwholesome and unfit for human consumption. Several researches (Bakari *et al.*, 2015; Egbebi *et al.*, 2016; Fasanmi *et al.*, 2010) have been carried out to assess the abattoir practices in Nigeria and other developing countries. However, information on quality of meat in relation to the extrinsic factors such as contact surfaces and hygienic practices of the abattoir workers in Oyo state is insufficient.

Materials and Methods
The experiment was conducted in three different production outlets located in major cities; Ibadan, Ilora and Ogbomoso within Oyo state. The samples collected from these locations were taken to the laboratory for microbiological analysis.

Sample collection:
Samples of fresh beef, surface swabs and water were taken from three different slaughtering points between 9 a.m. and 10a.m.in each of the production outlets; Akinyele central abattoir, Atenda Slaughter house and Kara Sawmill slaughter slab Ilora. A total of 127 samples were collected aseptically (27 beef samples, 9 water samples, 27 surface swabs of knives, 27 surface swabs of cutlasses and 27 surface swabs of floors) from three different slaughter facilities. These samples were aseptically collected in sterile Ziploc polythene packs, sealed and transported in cool box with ice packs to the laboratory for microbiological analysis within 1-4 hours of collection. This exercise was repeated in three batches on a weekly basis for three weeks.

Sample preparation
Meat sample
One gram (1g) of the beef sample was weighed and aseptically taken into a sterile jar containing 9ml of sterile normal saline diluent. It was homogenized for 15 seconds and a 1 ml aliquot of homogenate was transferred to a test tube containing 9 ml sterile distilled water to make 10^{-1} dilution and well mixed. Serial dilutions up to 10^{-4} were prepared for the microbiological analysis (Fawole and Oso, 2001). Then 1ml of sterile culture media was poured into each sterile petri dish, distributed and mixed evenly throughout. The petri dishes with molten inoculated media were allowed to solidify. All samples inoculated in nutrient agar were incubated at 37ºC for 24 hours to get (TVC) while samples inoculated in MacConkey agar were incubated at 37ºC and 44ºC for 24 hours for Total Coliform Count (TCC) and Potato dextrose agar for Total Fungal Count (TFC) counts respectively (Bhandare *et al.,* 2009).

Surface swabs
The swab sticks were swirled gently in a sterile bottle containing 9ml of sterile water. The aliquots were taken using sterile syringe and further diluted serially (10^{-4} folds dilution) into 10 test tubes. The diluents were mixed well and then one millilitre of diluted sample was poured into various sterile petri dishes and covered with one millilitres of sterile nutrient agar. Each plate was swirled gently taking care not to spill its contents and allowed to set. All samples inoculated with nutrient agar was incubated at 37ºC for 24 hours for TVC while samples inoculated in MacConkey agar were incubated at 37ºC for 24 hours for TCC (Bhandare *et al.,* 2009).

Water samples
A serial dilution of water sample was done into several test tubes. One milliliter of inoculum was taken from the test tube using a syringe and poured into sterile petri dish. Then 1 ml of sterile nutrient agar or MacConkey agar was poured into sterile petri dish, distributed and mixed evenly throughout the petri dish and allowed to solidify. All samples inoculated in nutrient agar were

incubated at 37ºC for 24 hours for TVC while samples inoculated in MacConkey agar were incubated at 37ºC for 24 hours for TCC and (Bhandare *et al.,* 2009).

Microbiological analysis
Total Viable Count (TVC)
Total Viable Counts were isolated and enumerated by pour plate method and grown on Nutrient Agar (NA). Serial dilutions of up to 10^{-4} were prepared by diluting 1g of the sample into 9ml of sterilized distilled water. One milliliter (1ml) aliquots from each of the dilutions were inoculated into Petri dishes with already prepared NA. The contents were swirled gently to thoroughly mix the agar with the inoculums. The plates were then inverted and incubated at 37ºC for 24 hours. After incubation all white spot or spread were counted and recorded as total viable count.

Total Coliform Count (TCC)
Total Coliform Counts were isolated and enumerated by pour plate method and grown on Mac Conkey Agar (MCA). Serial dilutions of up to 10^{-4} were prepared by diluting 1g of the sample into 9ml of sterilized distilled water. One milliliter (1ml) aliquots from each of the dilutions were inoculated into Petri dishes with already prepared MCA. The contents were thoroughly mixed. The plates were then inverted and incubated at 37ºC for 24 hours.

Enumeration of Staphylococcus species
Staphylococcus species were isolated and enumerated by pour plate method and grown on Salt Mannitol Agar (SMA). Serial dilutions of 10-1 to 10^{-4} were prepared by diluting 1g of sample into 9ml of sterilized distilled water. One milliliter aliquots from each of the dilution were inoculated into Petri dishes with already prepared SMA. The inoculum was evenly spread and allowed to dry for 15 minutes at room temperature. The plates were inverted and incubated at 37 ºC for

24 hours. After incubation yellow colonies were counted and recorded as *Staphylococcus* counts.

Enumeration of Escherichia coli
Escherichia coli were isolated and enumerated by pour plate method and grown on MacConkey agar. Serial dilutions 10^{-1} to 10^{-4} were prepared by diluting 1g of beef sample into 9ml sterilized distilled water. One milliliter aliquots from each of the dilution were inoculated into Petri dishes with already prepared MacConkey agar. The plates were then incubated at 37ºC for 24 hours. After incubation *Escherichia coli* pink colonies were counted and recorded as *E. coli* counts.

Enumeration of Salmonella
Prepared 10 ml of manufactured formula of Buffered peptone water (BPW), Oxoid CM009 (containing peptone 10.0; sodium chloride 5.0; pH 7.2 ± 0.2 at 25 ºC) was in a universal bottle and serial dilution of samples added to it. It was incubated at 37 ºC for 24 hours. Then 0.1 ml of the sample from the BPW was placed in a 10 ml of serenite broth in universal bottle and incubated at 44 ºC for 48 hours. *Salmonella- Shigella* agar (SSA) was added and incubated for 48 hours at 37ºC. Cream colonies with black centers on the SS agar indicated the presence of *Salmonella.*

Enumeration of Fungi
The pour plate method was used. One gram of the sample was poured into 9ml of sterile distilled water and mixed thoroughly by shaking. This was further diluted to obtain 10^{-4} concentration. Then 0.1ml dilution was transferred from each dilution bottle into the corresponding plate and sterile Potato Dextrose Agars (PDA). The plate was shaken gently to mix the content properly, and then it was allowed to settle and subsequently incubated at 37ºC for 72 hours. After incubation the plates revealing colony forming units were counted and the plate counts were expressed as log

CFU/g.

Statistical Analysis

The data obtained from the microbial examination were subjected to One–way ANOVA using the SAS® Statistical Package version 9.4 (SAS Institute, Cary, NC). Plate counts were converted to base-10 logarithm values and subjected to analysis of variance using PROC GLM and Fisher Protected LSD in SAS version 9.4.

Results

The results presented in Table 1 indicated that there were no significant (P<0.05) differences in the TVC and TFC of the fresh beef collected across the slaughter facilities. However, significant differences exists (P<0.05) between the means of the TCC. There are significant differences (P<0.05) in the mean TVC, TCC and TFC of the water sample. Also, there were no growth for TEC, TSC and TSLC in the beef and water samples.

TEC, TSC and TSLC were recorded on the swabs taken from all the contact surfaces (knives, cutlass and floors) with the floor having the highest mean values. The mean $\log_{10}CFU/cm^2$ values of TEC for floor were 1.95, 2.00 and 1.75 while TSC ($\log_{10}CFU/cm^2$) were 1.72, 1.66 and 1.72 and TSLC ($\log_{10}CFU/cm^2$) were 1.62, 1.58 and 1.71 for Akinyele central abattoir, Kara Sawmill slaughter slab and Atenda slaughter slab respectively. Table 2 shows contamination of water used for carcass processing and significant differences for TVC, TCC, and TFC among the facilities. Akinyele had the highest water TVC (3.29) and TCC (2.77). Table 3 indicates the level of contamination of knives used in butchering activities across the facilities with significant differences recorded for TVC, TCC and TSC. Akinyele recorded the highest values of knife contamination for TVC (4.58) and TCC (3.46) while Atenda recorded the highest knives TSC (1.78). Table 4 shows cutlass

Table 1: Microbial flora ($Log_{10}cfu/g$) on beef from the selected slaughter facilities in Oyo state

Parameters	Location				
	Akinyele	Kara	Atenda	SEM	P-value
TVC	3.12[b]	3.37[a]	3.24[ab]	2.50	0.537
TCC	2.30 [a]	1.82 [c]	1.91 [b]	1.31	0.041
TFC	2.12[ab]	2.06[b]	2.43[a]	1.49	0.099
TEC	ND	ND	ND	-	-
TSC	ND	ND	ND	-	-
TSLC	ND	ND	ND		

[ab] Means along the row with different superscript are significantly (P<0.05) different .**TVC** = Total Viable Count, **TCC** = Total Coliform count, **TFC** = Total Fungal count, **TEC** = Total Escherichia count, **TSC** = Total Staphylococcus count, **TSLC** = Total Salmonella count, **Cfu/gm** = Colony forming unit, **SEM**= Standard Error of Mean, **ND**= Not Detected.

Table 2: Microbial flora (Log_{10}cfu/g) on water from the selected slaughter facilities in Oyo state

Parameters	Location				
	Akinyele	Kara	Atenda	SEM	P-value
TVC	3.29 [a]	2.47 [b]	2.58 [b]	2.27	0.014
TCC	2.77 [a]	1.79 [b]	1.80 [b]	1.53	0.000
TFC	1.55 [b]	1.66 [a]	1.56 [ab]	0.03	0.396
TEC	ND	ND	ND	-	-
TSC	ND	ND	ND	-	-
TSLC	ND	ND	ND	-	-

[ab] Means along the row with different superscript are significantly (P<0.05) different .**TVC** = Total Viable Count, **TCC** = Total Coliform count, **TFC** = Total Fungal count, **TEC** = Total Escherichia count, **TSC** = Total Staphylococcus count, **TSLC** = Total Salmonella count, **Cfu/gm** = Colony forming unit, **SEM**= Standard Error of Mean, **ND**= Not Detected.

Table 3: Microbial flora (Log_{10}cfu/g) on knives from the selected slaughter facilities in Oyo state

Parameters	Location				
	Akinyele	Kara	Atenda	SEM	P-value
TVC	4.58 [a]	3.93 [b]	3.64 [b]	3.62	0.012
TCC	3.46 [a]	2.76 [c]	3.11 [b]	2.37	0.008
TFC	2.69 [ab]	2.81 [a]	2.60 [b]	1.88	0.470
TEC	1.74 [a]	1.53 [b]	1.70 [ab]	0.64	0.280
TSC	1.28 [c]	1.55 [b]	1.78 [a]	0.56	0.030
TSLC	ND	ND	ND	-	

[ab] Means along the row with different superscript are significantly (P<0.05) different .**TVC** = Total Viable Count, **TCC** = Total Coliform count, **TFC** = Total Fungal count, **TEC** = Total Escherichia count, **TSC** = Total Staphylococcus count, **TSLC** = Total Salmonella count, **Cfu/gm** = Colony forming unit, **SEM**= Standard Error of Mean, **ND**= Not Detected.

Table 4: Microbial flora (Log_{10}cfu/g) on cutlasses from the selected slaughter facilities in Oyo state.

Parameters	Location				
	Akinyele	Kara	Atenda	SEM	P-value
TVC	4.36[a]	4.40[a]	4.17[ab]	3.66	0.755
TCC	3.76[a]	3.43[b]	3.44[b]	2.59	0.056
TFC	3.42[ab]	3.05[b]	3.44[a]	2.49	0.176
TEC	1.67[ab]	1.76[a]	1.44[b]	0.62	0.128
TSC	1.57[a]	1.56[a]	1.56[a]	0.59	1.000
TSLC	ND	ND	ND	-	-

[ab] Means along the row with different superscript are significantly (P<0.05) different .**TVC** = Total Viable Count, **TCC** = Total Coliform count, **TFC** = Total Fungal count, **TEC** = Total Escherichia count, **TSC** = Total Staphylococcus count, **TSLC** = Total Salmonella count, **Cfu/gm** = Colony forming unit, **SEM**= Standard Error of Mean, **ND**= Not Detected.

Table 5: Microbial flora (Log_{10}cfu/g) on floors from the selected slaughter facilities in Oyo state

Parameters	Location				
	Akinyele	Kara	Atenda	SEM	P-value
TVC	5.18[a]	5.11[ab]	4.73[b]	4.31	0.282
TCC	4.62[a]	4.05[b]	3.97[b]	3.46	0.001
TFC	3.78[ab]	3.67[b]	4.01[a]	3.22	0.394
TEC	1.95[ab]	2.00[a]	1.75[b]	1.55	0.457
TSC	1.72[a]	1.66[ab]	1.72[a]	0.63	0.774
TSLC	1.62[ab]	1.58[b]	1.71[a]	0.54	0.513

[ab] Means along the row with different superscript are significantly (P<0.05) different .**TVC** = Total Viable Count, **TCC** = Total Coliform count, **TFC** = Total Fungal count, **TEC** = Total Escherichia count, **TSC** = Total Staphylococcus count, **TSLC** = Total Salmonella count, **Cfu/gm** = Colony forming unit, **SEM**= Standard Error of Mean, **ND**= Not Detected.

contamination, where significant differences were observed for only TCC and TFC. Akinyele had significantly higher values for both parameters. Table 5 shows floor contamination across the facilities, and only TCC had significant differences for this parameter. Also following earlier observed trend, Akinyele had the highest TCC value (4.62) among the selected facilities.

Discussion
Total viable count on meat samples collected from the slaughter facilities during the study did not exceed the accepted range of (> 5.0 $\log_{10}$cfu/g) according to FAO (2007). Hence, meat sample obtained from all the facilities were fit for human consumption. The values obtained in the study were lower when compared to what was reported by Hailese-lassie *et al.* (2012) in which the mean TVC values of microbial load of abattoir meat and butcher shops meat were 5.04 and 5.75 Log_{10} cfu/g. Difference in the bacterial counts across the slaughter facilities visited could be due to the differences in the level of hygiene of butchers and the initial health status or condition of the animals prior to slaughter.

Total coliform count on meat samples from this study did not exceed the recommended set standard of coliform bacteria counts (< 3.0 $\log_{10}$ cfu/g) according to (FAO, 2007). The findings in this study were similar to the result of Paul and Sylvia (2014) who reported that Coliforms counts were lower on meat at the abattoir (2.7 $\log_{10}$cfu/g) than at the butcheries (6.70 $\log_{10}$cfu/g). Fazlina *et al.* (2012) also found lower contamination of meat with Coliforms at the abattoir level.

Escherichia coli was not isolated in the meat samples which implies that the meat were probably well washed in water free from *Escherichia coli* and did not come in contact with faecal matter. Staphylococcus count was not also detected in the meat samples and this

disagrees with studies by other researchers that found a high prevalence of *Staphylococcus aureus* in raw meat and abattoir (Ahmad *et al.,* 2013; Soyiri *et al.,* 2008). Fazlina*et al*. (2012) also found very low levels of *Staphylococcus aureus* contamintion of meat at the government abattoirs. Similarly, Paul and Sylvia (2014) found low contamination of meat at the abattoir with *Staphylococcus aureus* count of 3.43 $\log_{10}$cfu/g compared to the contamination at the butcheries (5.73 $\log_{10}$cfu/g), though both values were higher than the standard limit for *Staphylococcus aureus* (PFA rules 2004) which is 2.01 $\log_{10}$cfu/g.

The fungal count from these findings was lower than that reported by Omorodion and Odu (2014) which revealed that the total fungal count ranged from 4.78 $\log_{10}$cfu/g to 5.64 $\log_{10}$cfu/g. Olufunmilayo and Akeeb (2010) also reported that the mycoflora content of the meat samples ranged from 5.81 to 6.34 log cfu/g.

The mycoflora on the fresh meat may have been derived from contaminants in water, air, floors and surroundings of the slaughter house.

Total viable count in water was lower when compared to what Adeyemo *et al*. (2002) reported which revealed that the TVC of 4.3 logs CFU/ml was found in water used at the main abattoir in Ibadan. The low total viable count in water is an important factor contributing to the low level of bacteria count in the meat. Tarwate *et al*. (1993) reported lower mean values for TVC compared to what was reported in the present study in water which was 2.1 log CFU/cm^2

The highest total coliform count mean values on water samples were observed in Akinyele Central abattoir. This is an indication that the level of faecal contamination is high which

maybe as a result of the slaughter of large numbers of animals. A similar study was done by Tarwate *et al.* (1993) where higher values of Enterobacteriaceae were reported on water (4.4 log cfu/cm^2).

E. coli, *Staphylococcus* and *Salmonella* counts were not recorded from the water samples collected in the study. However, TEC, TSC and TSLC were recorded on the swabs taken from all the contact surfaces. The highest mean values of total fungal count in water was observed in Kara sawmill slaughter slab with mean values (average for all the three batches) of 1.66 log cfu/ml. The presence of fungi in the water from the slaughter facility may be as result of dirty containers used in fetching the water from the well or fungal growth on the rims of the well.

Sources of microbial contamination
The main source of *Staphylococcus aureus* in the slaughterhouse was identified to be, the knives, cutlass and slaughtering floor. Staphylococci are ubiquitously distributed in man's environment and strains present in the nose often contaminate hands, fingers, and face, hence the isolation of the pathogen from the personnel coming in contact with equipment during this study (Gill, 2007). Faecal matter contamination, Sneezing, handling the meat with unwashed hands, handshake, handling money while in food production and processing area may be responsible for the propagation of bacteria to the equipment during this study (Gill, 2007). The cracks on the slaughter floors are also possible sources of fungi as fungi is associated dry places, cracks and crevices.

Conclusion
The results obtained from this study showed that there was lower microbial load on the meat when compared to that of the equipment used at the different slaughter facilities. The higher microbial log mean values (TVC, TCC and TFC) from the contact surfaces examined are indications of poor personal hygiene and sanitary practices and making them potential sources of food borne infection and food spoilage. Also, the absence of certain microbes such as *Escherichia coli*, *Staphylococcus* spp and *Salmonella* spp in the meat samples collected in this study is not complete guarantee of the absence of such pathogens in subsequent times since those pathogens were isolated from the equipment and floor. Stricter adherence to hygiene principles like: regular sterilization of knives, cutting equipment, and greater attention to personal hygiene should be followed by butchers in Nigeria to reduce the risks of a public health catastrophe.

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