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Assessment of beef meat microbial contamination during skinning, dressing, transportation and marketing at a commercial abattoir in Kigali city, Rwanda

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ABSTRACT

The study was conducted to assess the bacteriological contamination of beef meat in a commercial abattoir at slaughtering stages (skinning and dressing), during transportation from the abattoir to butcheries and during marketing in Kigali City. Twenty four samples were collected (6 samples at each stage) and the total bacterial, total coliforms, *Escherichia coli* and *Staphylococcus aureus* counts enumerated using conventional microbial plate culture methods. The results showed the contamination of carcasses by all tested bacterial groups except *S.aureus*. The level of microbial contamination increased progressively after the slaughtering of cattle to the marketing of carcasses. The contamination by total aerobic bacteria increased from 5.1 to 10.9 log CFU/g. While contamination by total coliforms increased from 3.1 to 4.7 log CFU/g and the contamination by *E. coli* increased from 0.8 to 3.0 log CFU/g. *S.aureus* was not detectedat all the four considered stages. Compared to the European Microbiological Standards for meat, the observed levels of beef carcasses contamination, from the skinning stage to the marketing level, were found to be out of the acceptable range. This could be due to contamination at slaughtering, transportation and marketing stages. In addition, handling meat at ambient temperatures could have lead to increased microbial load during transportation and marketing. Therefore, there is need to improve on hygiene during slaughtering, marketing and transportation in Kigali City.

Keywords: Beef meat, microbiological contamination, slaughtering, transportation, marketing

INTRODUCTION

The beef meat contains 70-73% of water, 20-22% of protein and 4.8% of lipids (Alan *et al.*, 1995). This chemical composition exposes beef meat to the contamination by spoilage and pathogenic bacteria when adequate hygienic measures during the preparation, transport and marketing are not respected (Hudson *et al.*, 1996). In most developing countries, the absence or non respect of the existing hygienic practices in slaughtering, transportation and marketing has been found to be one of the major causes of meat contamination by pathogenic and non pathogenic microorganisms (FAO, 2004). Adzitey *et al.* (2011) reported that in Ghana a number of abattoirs and meat processing units do not meet sanitary standards and operated without adequate quality control systems. In many developing countries, meat is normally transported to markets either in vans, motocycles and at times even using bicycles. In most instances the transportation systems are made of surfaces that are difficult to clean and disinfect. Furthermore, meat are sold in the open markets on tables that are not well cleaned and disinfected. Thus exposing meat to a number of microorganisms which may be pathogenic or non pathogenic.

In Rwanda there is no scientific data available addressing the microbiological quality of meat during the slaughtering and transportation. However a study conducted by Hirwa (2010) showed that beef sold in selected markets of the District of Nyarugenge (Kigali, Rwanda) was out of European Union microbiological standards. All analyzed beef samples were out of acceptable limits for total aerobic bacteria and only 26.6 % and 10 % of the analyzed samples were within the acceptable range respectively for *Escherichia coli* and thermotolerant coliforms. Norman *et al.* (2006) indicated that the contamination of meat at the end consumers level, correspond to the combination of contaminations at different stages of meat preparation including the slaughtering, transportation and marketing. During the slaughtering process the stages of skinning and the dressing were identified to be the critical points for carcasses microbiological contamination (Gill *et al.*, 2003). In order to quantify the contribution of different processing stages on the final contamination of meat, the present study was carried out to assess microbiological contamination of beef carcasses at different stages of meat preparation namely; skinning and dressing in slaughterhouse, during the transportation and at carcasses marketing stage. Four microbiological parameters were considered in this study: Total Aerobic Bacteria, Total coliforms, *Escherichia coli* and *Staphylococcus aureus*.

MATERIAL AND METHODS

Description of slaughtering, meat transportation and marketing in Kigali city

The slaughtering, transportation and marketing process were scrutinized in order to identify key stages at which microbiological contamination was likely to occur.

Slaughtering; slaughtering of cattle was carried out as described in the Table 1. Stunning was done by using a captive-bolt pistol (non-penetrating type) which was directly applied to the forehead of the animal. Immediately following stunning the animals were hoisted by one leg and bleed by cutting the major blood vessels of the neck. Carcasses were skinned by using combined horizontal and vertical methods (FAO, 1991). The thoracic viscera were removed from the carcass after sawing the sternum and cutting the trachea.

The stomachs and intestines were removed by cutting respectively between the esophagus and the stomach and the caecum and rectum. No duplicate ties were made at the esophagus – stomach and coecum –rectum junctions. The carcass dressing was done manually by using knives. The facilities for carcass refrigeration were present but during the period of the study beef carcasses were not refrigerated. Concerning sanitation and hygiene, it was noted that in the slaughterhouse there were hand-washing stations with running water but hand washing detergents and disinfectants was not present at all stations. There were no hand-washing stations with running water in the butcheries. The slaughterhouse had knife sterilizing stations but were not functioning at the period of the study. The slaughterhouse had fly screens to protect contamination of meat by flies. In the slaughterhouse the staff was provided with aprons but no head cover.

Table 1: Beef carcasses preparation stages at the Kigali commercial abattoir

Serial number	Processing stages
1.	Stunning
2.	Bleeding
3.	Deheading and Legging
3.	Skinning*
4.	Evisceration
5.	Splitting
6.	Dressing*
7.	Refrigeration
8.	Transportation*
9.	Marketing*

*Stages at which sampling was done

Meat transportation; Carcasses were directly transported to the butcheries by simply heaping once carcass one on another and covering with a plastic sheet. The carcasses were transported in non refrigerated vehicles.

Marketing; In the butcheries, carcasses were suspended on hooks and meat pieces were exposed on tables. The butcheries had fly screens to prevent contamination of the meat by flying insects. In the butcheries, staffs were provided with both aprons and head covers.

Sample collection

A total of twenty four beef round steak samples from twenty four carcasses were randomly collected. Twelve samples were obtained during slaughtering (six samples at the stage of skinning and – six samples at the dressing stage), six samples were collected during transportation and finally six samples from three butcheries located nearby the slaughterhouse (about 500 m from the slaughterhouse). Samples were aseptically collected in sterile polythene bags, sealed and transported in an ice box to prevent microbial growth during sample transportation. The samples were analyzed immediately upon arrival in the laboratory.

Enumeration of bacteria

Sample preparation and serial dilution; a portion of beef (10 g) was minced in 90 ml of peptone water (Biolab – Merck, Wadesville, Hungary). The composition of the peptone water was as follows (g/l); Peptone 10.0, Sodium Chloride 5.0, di-Sodium Hydrogen Phosphate:3.5, Potassium di-Hydrogen Phosphate: 1.5 with the pH of 7.0. Ten-fold serial dilutions of the homogenized meat samples were performed using peptone water as diluent. One hundred micro liters of each dilution was inoculated into Petri dishes.

Aerobic Plate Count

Nutrient agar (Pronadisa, Madrid, Spain) was used for enumeration of total aerobic bacteria in the meat samples. The composition of the nutrient agar was as follows (g/l); Gelatin Peptone: 5.0, Beef Extracts: 3.0, Bacteriological Agar: 15.0 with the pH of 6.8. After sterilization at 121˚C for 15 mins, the media was cooled down. About 30 ml of the cooled media was poured into sterile petri dishes and immediately 100 µl was inoculated. The content in the petri dish was gently swirled clockwise and anticlockwise to thoroughly mix the media with the sample. The plates were inverted and then incubated at 30ºC for 48 hours.

Staphylococcus aureus

Baird-Parker agar (Pronadisa, Madrid, Spain) with the following composition (g/l); Glycine: 12.0, Casein Pancreatic Digests: 10.0, Sodium Pyruvate:10.0, Beef Extracts:5.0, Lithium Chloride: 5.0, Yeast Extracts:1.0, Bacteriological Agar: 20.0 with the pH of 6.8; was used for enumeration of *S. aureus*. An amount of 0.1 ml of each dilution of the sample was inoculated to the surface of Baird-Parker agar plates using the spread plate technique. The inoculum was evenly spread on the surface of the agar and allowed to dry for 15 min at room temperature. The plates were inverted and incubated for 24 ± 3h and re-incubated to a total of 48 ±4 h at 37ºC. Typical colonies of *S aureus* were black

or grey, shinning, convex and surrounded by a clear zone.

Total coliforms and *Escherichia coli* count

Mackonkey Agar (Biolab – Merck, Wadesville, Hungary) with the following composition (g/l); Peptone: 20.5; Bile Salts: 1.5; Lactose: 10.0, Sodium Chloride: 5.0, Neutral Red: 0.03, Cristal Violet: 0.001, Bacteriological Agar: 15.0 with the pH of 7.1; was used for enumeration of both total coliforms and *E. coli*. The method of inoculation and plating was the same as described for aerobic plate count. The plates were inverted and then incubated at 37ºC for 24 hours. The suspected colonies of *E. coli* appeared purple on Petri dishes after incubation at 37ºC for 24 hours.

Determination of microbial counts

Colonies on selected plates were counted using a colony counter (Bibby Scientific Limited, Staffordshire, UK). The morphological characteristics of each colony were examined to indicate the shape, size elevation and pigmentation to facilitate the process of grouping and identification. The determination of colony forming units (cfu) was performed by using the following formula (AFNOR,2001):

$$N = \frac{\sum c}{V(n_1 + 0{,}1\, n_2)d}$$

Where, N = total number of microorganisms present in one gram of the product; $\sum c$ = the sum of the colonies counted on all Petri dishes of two successive dilutions, V = the quantity inoculated on each Petri dish in milliliters; n_1 and n_2 = the number of considered Petri dishes respectively at the first and the second dilution and finaly d = the considered first dilution.

RESULTS AND DISCUSSION

The study on microbial contamination of fresh beef carcasses during the slaughtering process, transportation and marketing at a commercial abattoir of Kigali, was conducted mainly to assess the evolution of the microbial contamination of beef during its preparation process and to observe the use of hygienic practices which may reduce incidences of cross contamination in the slaughterhouse and the marketing area. The results of microbial analysis are summarized in the Table 2.

In the four considered stages (skinning, dressing, transportation and marketing) the total aerobic counts ranged from 5.1 to 10.9 log cfu/g. There were significant ($p \leq 0.05$) increases in total aerobic count during dressing, transportation and marketing. In between skinning and marketing there was a 5.8 log cfu increase in total aerobic bacterial counts indicating that a lot of contamination occurs during meat slaughtering, transportation and marketing in Kigali city.

Table 2: Averages of beef meat microbial contamination at different stages in log cfu/g

Processing stages	Total plate count	Total coliforms	*Escherichia coli*
Skinning	5.1±0.9 [a]	3.1±0.5 [a]	0.8±3.5 [a]
Dressing	8.1±1.5 [a]	3.5±1.7 [a]	2.1±0.7 [a]
Transportation	8.9±1.0 [b]	3.9±0.5 [a]	2.3±0.7 [a]
Marketing	10.9±1.1 [c]	4.7±0.8 [a]	3.0±0.6 [a]

[a,b,c] Mean values in the same column with different superscript are significantly ($p < 0.05$) different.

Total coliforms ranged from 3.1 to 4.7 log cfu/g representing 1.6 log cfu increase in between slaughtering and marketing of beef. The highest increase (0.8 log cfu) in total coliform counts occurred between transportation and marketing. The contamination by *E. coli* ranged from 0.8 to 3.0 log cfu/g. *S. aureus* was not detected at all stages. Compared to the European Microbiological standards for meat (CE, 2005), the observed levels of beef carcasses contamination, from the skinning stage to the marketing level, were found to be out of the acceptable range. The European Union recommends that the levels of contamination by total aerobic bacteria and total coliforms do not exceed respectively 5.0 and 2.5 log CFU/g. The pathogenic bacteria could be absent from meat. During our study, it was found that for all detected microorganisms except *Staphylococcus aureus*, the level of contamination was low at the skinning stage and increased progressively during the dressing, transportation and marketing stages.

The contamination of carcasses at the skinning stage could be due to the contact between the carcass and the hide. It has been reported that muscle tissue from uneviscerated carcass is sterile (Gill *et al.*, 1978). During skinning, the contact between carcass and hide allows a mixture of micro-organisms to be introduced onto the carcass (Bell, 1997). These contaminating microorganisms derive from the pre-slaughter environment and may be of faecal, soil, water or feed origin. Our results are in agreement with McEvoy *et al*. (2000) who reported that the contamination of beef carcasses in a commercial abattoir is correlated to the cleanliness of hides. Equipments like knives used in dehiding operations have been reported to be responsible for cross contaminations from one carcass to another or from personnel to carcasses especially when the facilities to sterilize knives after being used are lacking or not functioning accordingly. The contamination by coliforms could be due to the lack of hygiene. This is in agreement with Soyiri *et al*. (2008) who reported that the presence of coliforms and *E. coli* was as a result of meat contamination with faecal mater which could be from the environment, air, material used including water. The study conducted by *Elder et al*. (2000) in a cattle slaughtering facility also highlighted the clear correlation of Enterohemorragic *E.coli* (EHEC) O157 prevalence in feces, hides and carcasses. The hands of handlers could also be

implicated. Nel *et al*. (2004) reported that the lack of personal hygiene in an abattoir contributes actively to the contamination of meat especially by coliforms.

At the dressing stage the levels of contamination by all detected microorganisms was higher than the levels of contamination observed at the skinning stage. This could be due to the additional contamination of the carcass by microorganisms from the digestive tract. Our results are in agreement with Soyiri *et al.* (2008) who identified the gastrointestinal tract as a potential source of carcass contamination because of its high microbial load. This contamination was highly accentuated when the stomachs and intestines are punctured during the evisceration process (FAO, 2004; Gill *et al.*, 1996 ; Sheridan, 1998). The high level of coliforms especially *E. coli* are due to the fact that these microorganisms are also found in the animal's digestive tract (McEvoy *et al*., 2003).

A range of carcass intervention treatments have been designed to reduce the contamination of carcasses during the slaughtering process. These include; the low pressure hot water spray, high pressure water spry, steam pasteurization, acetic acid spray, irradiation, amongst others (Chen *et al.*, 2012).These carcass treatments were found to be effective in the reduction of spoilage and pathogenic microorganisms on carcasses in the slaughterhouse (Algino *et al.*, 2007; EFSA, 2011; Spoto *et al*., 2000) but their utilization in most of developing countries is still limited (FAO, 2004). In the studied slaughterhouse there was no antimicrobial treatment applied to carcasses and this could explain the high levels of carcass contamination observed at the transportation and marketing stages.

The levels of carcass contamination observed at the transportation stage were higher compared to the ones at the skinning and dressing stages. The additional contamination could have been due partially to the use of non refrigerated vehicles for transportation of beef carcasses which facilitate the multiplication of pathogenic and spoilage bacteria. Our results are in agreement with Nynchas *et al.* (2008) and the FAO – Good Practices for Meat Industry (2007) who reported that the warm temperatures during transportation encourage the growth of initial microflora and microorganisms got during different slaughtering steps. During the transportation from the abattoir to the butcheries, carcasses were simply heaped one upon another and covered with a plastic sheet. This unhygienic transportation mode encourages cross contamination and could have actively contributed to the observed contamination. The observed high levels of contamination by coliforms and *E. coli* could have been due to the growth of the existing microorganisms encouraged by the warm temperatures and the cross contamination from the transportation vehicle to carcasses or from a carcass to another.

In our study, the highest levels of carcass contamination were observed at the marketing stage. The additional contamination could have been due partially to the contamination of carcasses and meat cuts by undisinfected tables and the handling of meat with unsterilized instruments such as knives. This could be the consequence of the lack of knife-sterilizing equipments observed in the visited butcheries. Our results are in agreement with Adzitey *et al.* (2011) who identified the use of unsterilized instruments as the major source of meat contamination in Ghanaian markets. The hands of butchers could also be considered as contributors to the observed contamination. In the visited butcheries there were no hand-washing stations with running water and this could encourage the transfer of microorganisms from handlers to carcasses or from a carcass to another. Simiarily a study conducted by Nel *et al.* (2004) in South Africa showed that the lack of personal hygiene was the major factor contributing to the contamination of meat especially by coliforms. The contamination by coliforms and *E. coli* could also have been due to the growth of microorganisms that had contaminated the carcasses during the previous processing stages given that there was no decontamination interventions applied to carcasses at the slaughterhouse where the study was conducted. This is supported by the study conducted by McEvoy *et al.* (2003) at a commercial Irish abattoir that showed that the faecal contamination of carcasses by *E. coli* O157:H7 from hides and rumen occurring during hide removal and bung tying, remains during the subsequent washing, chilling and boning operations.

The contamination of carcasses increased progressively during the cattle slaughtering process, transportation and marketing of beef carcasses and meat cuts. The initial contaminating microorganisms both pathogenic and non pathogenic could have originated from the hide and the gastrointestinal tract of slaughtered animals. The high levels of carcass contamination by spoilage microorganisms deteriorate the quality of meat and decrease its shelf life (Soyiri *et al.*, 2008), hence the negative economic effect to meat processors. Also beef has been implicated for a number of meat borne infections and intoxications in several countries (Adzitey *et al*., 2011; Chen *et al*., 2012). Even if the mastery of some sources of meat contamination requires sizeable financial means, the rigid application of good hygienic practices during the slaughtering, transportation and marketing of beef carcasses can considerably reduce the contamination of meat in developing countries (FAO, 2004).

CONCLUSION

The study conducted showed that the contamination of beef carcasses increased progressively from the skinning stage in the slaughterhouse to the marketing area. The general sanitary conditions in butcheries in addition to the poor hygienic practices by meat handlers during the slaughtering, transportation and marketing are probable contributors to the microbial contamination on the beef. It is therefore highly recommended to the slaughterhouse and meat sellers to

rigidly enforce standard hygienic practices in the slaughtering, transport and marketing of meat carcasses to assure the quality and the safety of meat. The study looked at only four bacteria and did not address the identification of isolated microorganisms. There is a need for further studies to look at the evolution of the beef carcasses contamination by other pathogenic microorganisms and carrying out their advanced identification.

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