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Evaluation of microbial adverse effects on fresh and processed bovine meat in N'Djamena (Chad) and Yaoundé (Cameroun)

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A study from May 2015 to February 2016 was conducted in N'Djamena and Yaoundé regions to assess the microbial hazards associated with fresh and transformed bovine meat. A total of 120 samples of dried, fresh and spicy dry meat (*kilichi*) were collected. The microbiological results showed that 70% of dried meat from N'Djamena had *Escherichia coli* as major contaminant compared to 55% of dried meat from Yaoundé. 60% of fresh meat from Yaoundé was out of standards because it contained *Staphylococcus aureus* against 40% of fresh meat from N'Djamena and 25% of *kilichi* meat from Yaoundé containing *Salmonella;* it was only 10% of *kilichi* meat from N'Djamena that was positive for the same. The results of 70% of dried meat samples from N'Djamena as well as 70% of *kilichi* meat from Yaoundé had good standards with *S. aureus.* This work provides evidences that there is various food borne pathogenic bacteria in fresh and processed bovine meat sold in N'Djamena and Yaoundé, which is an indication of poor standards. It reinforces the urgent need for implementation of a quality management system in these sectors in Yaoundé and N'Djamena.

Key words: Microbial adverse effect, bovine fresh meat, dry meat, *Kilichi*, N'Djamena, Yaoundé.

INTRODUCTION

There are several reports of food borne illnesses related to consumption of meat, and hence the quality of meat and meat products is significant for public health. In 2009, the estimate of beef production was 67.5 million tons and red meat production was 277 414 tons in Central African sub regions such as Cameroon, Chad, Congo and Gabon (FAO, 2013; Faye et al., 2013). Meat is sold in these countries in fresh or processed form; for example,

dried meat (*charmout* in N'Djamena), processed and seasoned meat (*kilichi*) and smoked meat. About 25% of the population suffering from chronic malnutrition is from sub-saharan Africa (FAO, 2014). In Central Africa, meat consumption is important in reducing malnutrition rate; unfortunately the hygienic quality is not guaranteed (Raoul et al., 2015; Tidjani et al., 2013). Meat has a high protein content, with good biological value and contains all the essential amino acids, iron, zinc and vitamins A, B12, B6, D and E (Nfor et al., 2014). Majority of morbidity and mortality related foodborne infections have been caused by bacterial agents. Bacterial gastrointestinal infections continue to cause illness and death and contribute to great economic loss in most parts of the world. In industrialized countries food-borne illnesses have been reported in up to 30% of the total population (WHO, 2011). The problems caused by zoonotic microorganisms to public health are important in sub-Saharan Africa (Nicoline et al., 2015; Ternhag et al., 2008). Food-borne pathogens in meat find their source from meat processing, transportation and poor hygiene management of slaughterhouse. Therefore, analyzing pathogenic bacteria in meat is a standard practice to ensure safety and quality of meat. Cattle themselves and the environment where they are kept are the most important sources of pathogenic microorganisms and they may be the origin of contamination of meat and meat products (Mueen et al., 2003). Meat can also be contaminated by an unhealthy environment, dirty hands of manipulators, polluted water and soil (Raoul et al., 2015). Studies on meat carcasses have revealed the presence of pathogenic germs (Pradeep et al., 2014; Obeng et al., 2013). *Staphylococcus aureus* producing exotoxins and enterotoxins can also contaminate several foods, including processed meat products and cause staphylococcal food poisoning (Angel et al., 2014). *Escherichia coli*, as an enteric pathogen is increasingly important as potential foodborne pathogens for public health. *Salmonella* is also potentially important as causative of typhoid fever, gastroenteritis and diarrheal diseases (Doaa and Salwa, 2012). This work aims at assessing the level of contamination and distribution of some foodborne pathogens in fresh and processed bovine meat in N'Djamena (Chad) and Yaoundé (Cameroon) and the level of conformity to existing standards.

MATERIALS AND METHODS

Sampling

A total of 120 samples of dried and fresh meat and *kilichi* were collected from May 2015 to February 2016 from different points of sale in Yaoundé and N'Djamena. Twenty samples were collected per meat type from the meat production chain of the slaughterhouses, from each town. About 300 to 500 g of different bovine meats was sampled according to aseptic rules (NF ISO 18593, 2004). Samples of meat were wrapped in aluminum foil and kept in sterile plastic bags. They were clearly labeled, identified and

transported with ice pack (0 and 4°C), in a cooler (Keep Cold®) to the laboratories for immediate analysis. Microbial counting, isolation and identification of microorganisms were performed according to the criteria of EU Regulations and Specific Standards (EC) N° 1441/2007 on meat.

Microbiological analysis

In the laboratory, for each sample, 10 g was introduced aseptically into a sterile stomacher bag and macerated in 90 ml of sterile diluent (0.1% peptone and 0.8% sodium chloride from *DIFCO LABORATORIES Detroit MI USA*). 1 ml of the suspension was diluted into 9 ml of sterile diluent and used for cultivation of micro-organisms. Various dilutions were done in accordance with the standards of NF EN ISO 6887, 2011. Routine enumeration and identification of foodborne pathogens including *Salmonella, Staphylococcus aureus* and *Escherichia coli* in meat samples were carried out by conventional methods. Identification of suspicious colonies of *Salmonella* and *E. coli* was made by biochemical reactions with *Biomerieux API®20E* galleries, REF *20100 CE* (Institute of Livestock Research for Development of N'Djamena).

About 0.1 ml of sample from appropriate dilutions was plated on the following culture media (all from *Biolife*): Total aerobic mesophilic bacteria were counted on plate count agar after incubation at 30°C for 72 h (ISO 4833-1, 2013). Violet Red Bile Glucose Agar plates, incubated at 37°C for 24 h, were used to count Enterobacteriaceae (NF ISO 21528-2: 2004). *E. coli* was enumerated at 44°C for 24 h on Tryptone Bile X-glucuronide Agar plates (NF ISO 21528-2: 2004). Coagulase - positive staphylococci were enumerated in Chapman Mannitol Salt Agar (from *Biolife*) at 37°C for 48 h (NF V08-057-1, 2004). *Salmonella* was investigated according to the standard method of NF ISO 6579 (2002). A portion of 25 g of meat was suspended in 225 ml of buffered peptone water for pre-enrichment. The homogenized solution was incubated at 37°C for 24 h.

For secondary and selective enrichment, 0.1 ml of the suspension was added to 10 ml of Rappaport Vassiliadis broth and incubated at 42°C for 24 h. The selective isolation was carried out on medium Xylose-Lysine-Deoxycholate agar and Hektoen agar. Typical colonies of the desired microorganisms were sown into nutrient agar at 37°C for 24 h for biochemical tests.

Biochemical tests

Biochemical tests were carried out using *API®20E* galleries. Gram strain, catalase and oxidase tests were carried out on the isolated colonies (*Salmonella* and *Escherichia*). The coagulase positive staphilococci were confirmed by Gram stain, catalase, oxidase, and coagulase tests (NF V08-057-1, 2004). 50 suspected bacterial colonies, 27 *Salmonella* and 23 *Escherichia* isolates were used for identification. The suspected bacteria were checked by the rapid urease test with urea indole *UI-F Biomerieux SA Ref. 55752 EC. Biomerieux API®20E* galleries *20100 CE* was used for biochemical identification of suspicious isolates of *Salmonella* and *Escherichia.*

The maximum concentrations accepted for the counted bacteria were as follows: Total Aerobic Mesophilic, 5.6 log10 cfu/g; Enterobacteriaceae, 3 log10 cfu/g; *E. coli*, 1.6 log10 cfu/g; *S. aureus,* 2 log10 cfu/g and the absence of *Salmonella in* fresh meat, according to the criteria of EU regulation and standards (EC) N° 1441/2007.

Statistical analysis

Data were analyzed using descriptive statistics with IBM SPSS 20 software. Analysis of the statistical recapitulative reports was

Table 1. Overall microbiological quality of different samples of meat (Average ± Standard Deviation, expressed as log10 cfu/g).

Microorganism	TAM	Enterobacteriaceae	*E. coli*	*S. aureus*	*Salmonella*
Meat type (n[1] =120)	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD
FM N'Djamena (n=20)	4.8 ± 0.4	4.3 ± 0.7	1.8 ± 1.5	1.5 ± 1	0.3 ± 0.7
FM Yaoundé (n=20)	3.9 ± 0.3	3 ± 1.1	2.1 ± 1.2	2.1 ± 1.3	1 ± 0.9
DM N'Djamena (n=20)	5.3 ± 0.8	4.2 ± 1.8	3.1 ± 1.7	2.1 ± 1	1.2 ± 0.9
DM Yaoundé (n=20)	3.6 ± 0.4	4.3 ± 6.8	2.2 ± 1.1	2.4 ± 0.9	1.1 ± 0.8
KM N'Djamena (n=20)	2.7± 1.6	0.9± 1.2	0.1± 0.4	0.8± 1.1	0.1± 0.4
KM Yaoundé (n=20)	3.5 ± 0.5	0.8 ± 1.3	0.7 ± 1	1.8 ± 1.2	0.5 ± 0.7

n = Number of samples analysed; n[1] = Total samples analysed; M, Mean; SD, Standard Deviation; CFU, colony forming unit; FM, fresh meat; DM, dry meat; KM, Kilichi meat; TAM, total aerobic mesophilic germ.
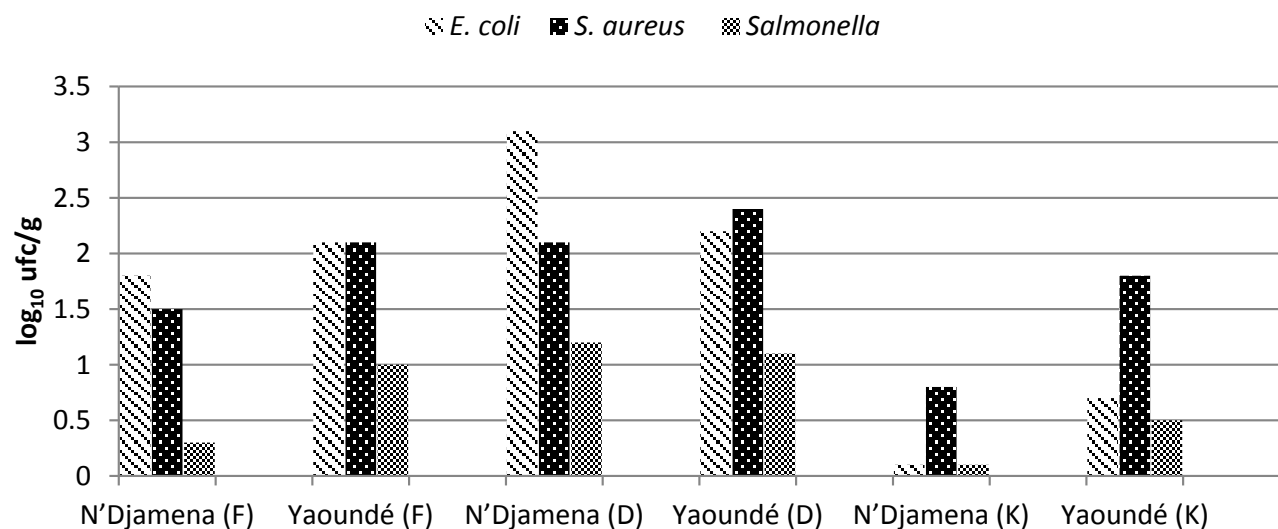
Figure 1. The average number of pathogenic bacteria for 20 samples per meat types and for each town. *F, Fresh meat; D, Dry meat; K, Kilichi meat.*

applied to compare the results of the enumeration between the means and standard deviation. Significance was determined at 5% confidence level. The results expressed in ufc/g were transformed to logarithmic decimal (log10 ufc/g) to normalize the data and discuss with the microbiological approach.

RESULTS

In Table 1, the average microbial count and standard deviation for TAM, Enterobacteriaceae, *E. coli, S. aureus and Salmonella* are presented for the 3 types of meat analyzed. It can be observed that for fresh meat, samples from Yaoundé had a microbial lead for total aerobic mesophilic (TAM) and Enterobacteriaceae which is lower than that of samples from N'Djamena. On the contrary, samples from Yaoundé were higher in *Staphylococcus* (2.1 $\log_{10}$ ufc/g) *and Salmonella* (1 $\log_{10}$ ufc/g) counts than those of fresh meat from N'Djamena. The microbial quality of dried meat was 5.3 $\log_{10}$ ufc/g for TAM and 3.1

$\log_{10}$ ufc/g for *E. coli* for samples from N'Djamena. In the case of *Kilichi,* it was observed that samples from N'Djamena were more contaminated than those from Yaoundé only for Enterobacteriaceae (Table 1).

The average number of bacteria obtained for the different meat types are represented in Figure 1 where the average microbial counts for *E. coli* ($\log_{10}$ ufc/g)*, S. aureus* ($\log_{10}$ ufc/g) and *Salmonella* ($\log_{10}$ ufc/g) are presented for fresh meat, dried meat and *Kilichi* from Yaoundé and N'Djamena. For *E. coli, Kilichi* samples from N'Djamena had a mean lower microbial count (0.1 $\log_{10}$ ufc/g). The mean number of *E. coli* (Figure 1) was very high for dried meat from N'Djamena (3.1 $\log_{10}$ ufc/g). The mean bacterial count of *S. aureus* was lower for *Kilichi* meat from N'Djamena (0.8 $\log_{10}$ ufc/g) than that of *Kilichi* from Yaoundé (1.8 $\log_{10}$ ufc/g). The mean count of *Salmonella* was lower for dry meat from Yaoundé than for the dried meat samples from N'Djamena (Figure 1).

Table 2 shows the numbers and percentages of

Table 2. Distribution of results according to critical limits.

Meat type (n^1 =120)	Microorganism	NS that satisfied standards		NS exceed limit*	
		N'Djamena	Yaoundé	N'Djamena	Yaoundé
Fresh meat	TAM	20 (100%)	20 (100%)	0 (0%)	0 (0%)
	Enterobacteriaceae	4 (20%)	6 (30%)	16 (80%)	14 (70%)
	E. coli	11 (55%)	7 (35%)	9 (45%)	13 (65%)
	S. aureus	12 (60%)	8 (40%)	8 (40%)	12 (60%)
	Salmonella	16 (80%)	12 (60%)	4 (20%)	8 (40%)
Dry meat	TAM	13 (65%)	20 (100%)	7 (35%)	0 (0%)
	Enterobacteriaceae	5 (25%)	13 (65%)	15 (75%)	7 (35%)
	E. coli	6 (30%)	9 (45%)	14 (70%)	11 (55%)
	S. aureus	14 (70%)	10 (50%)	6 (30%)	10 (50%)
	Salmonella	8 (40%)	9 (45%)	12 (60%)	11 (55%)
Kilichi meat	TAM	20 (100%)	20 (100%)	0 (0%)	0 (0%)
	Enterobacteriaceae	18 (90%)	20 (100%)	2 (10%)	0 (0%)
	E. coli	20 (100%)	20 (100%)	0 (0%)	0 (0%)
	S. aureus	19 (95%)	14 (70%)	1 (5%)	6 (30%)
	Salmonella	18 (90%)	15 (75%)	2 (10%)	5 (25%)

n^1 = Total samples analyzed; n = Number of samples analyzed; NS, number of samples; TAM, total aerobic mesophilic bacteria; *NS exceed limit, The number of samples that do not satisfy the standards according to the criteria of EU regulation, standards (EC) N° 1441/2007.

Table 3. API 20E strips galleries of Enterobacteriaceae identification system.

Microorganism	Suspected bacteria for *Salmonella* (n = 27)	Suspected bacteria for *E. coli* (n = 23)	Total number of bacteria
Salmonella spp.	14 (51.8%)	0 (0%)	14
Salmonella cholerae arizonae	1 (3.7%)	0 (0%)	1
E. coli	0 (0%)	15 (65.2%)	15
Enterobacter	4 (14.8%)	1 (4.3%)	5
Klebsiella	1 (3.7%)	1 (4.3%)	2
Citrobacter	2 (7.4%)	0 (0%)	2
Serratia	0 (0%)	1 (4.3%)	1
Hafnia	1 (3.7%)	0 (0%)	1
Erwinia	1 (3.7%)	0 (0%)	1
Undetermined	3 (11.1%)	5 (21.7%)	8

n = Number of suspected bacteria.

samples that do not contain microorganisms and those with exceeding microbiological limits. About 80% of fresh meat from N'Djamena contain Enterobacteriaceae which is greater than that for fresh meat from Yaoundé (70%). 60% of fresh meat from Yaoundé exceed limit standard of *S. aureus* against 40% of fresh meat from N'Djamena. The dried meat from N'Djamena contains more *E. coli* (70%) than dried meats from Yaoundé (55%). More than 50% of dried meat samples from N'Djamena and Yaoundé contained *Salmonella*. Meanwhile, 25% of *kilichi* from Yaoundé and 10% from N'Djamena contained *Salmonella*.

The results of identification of bacteria (API 20E strips galleries) for 50 suspected bacteria are represented in Table 3. The assay revealed 51.8% of *Salmonella* spp. and 3.7% of *Salmonella cholerae arizonae* in the meat samples. The percentage of *E. coli* among suspected bacteria (for *E. coli*) was 65.2% (Table 3). *Enterobacter, Klebsiella, Citrobacter, Serratia, Hafnia, Erwinia* and 8 other undetermined bacteria were also identified by API 20E strips galleries.

DISCUSSION

In general, the different types of bovine meat analyzed were contaminated with food borne pathogens (60% of fresh meat from Yaoundé exceed limit standard of *S.*

aureus, 40% from N'Djamena. 70% of dried meat from N'Djamena contain more *E. coli*; more than 15% of fresh meat samples from N'Djamena and Yaoundé contained *Salmonella*). For fresh meat, there was total aerobic mesophilic of 5.6 log10 cfu/g; Enterobacteriaceae, 3 log10 cfu/g; *E. coli*, 1.6 log10 cfu/g; *S. aureus,* 2 log10 cfu/g and absence of *Salmonella* according to the regulation and standards (EC) of N° 1441/2007.

These results corroborate with studies of Pradeep et al. (2014) which showed that 5% of the analyzed samples of meat from Karnataka region in India contain *Salmonella*, 6.6% contain *Staphylococcus*, and 7.3% have *E. coli* (Pradeep et al., 2014).

In Cameroon and Chad, fresh processed meats are usually kept at room temperature and this practice causes the proliferation of microorganisms. Deriba and Mogessie (2001) indicated that foods that are held at ambient temperatures of 15 to 45°C for more than 4 h present a considerable public health risk. In case of fresh meat, absence of clean water and washing facilities in the vending environment and lack of awareness of the vendors about food handling and safety issues might be responsible for these results (Table 2). Washing of hands has not been a common practice in Chad and Cameroun.

The pathogens isolated in the present study were similar to those reported by Fasanmi et al. (2010), Pradeep et al. (2014) and Anbessa (2013) where *Salmonella, E. coli, S. aureus* and *Listeria* were isolated from meat in Nigeria, India and Ethiopia. *E. coli,* in different amounts, were isolated from the various meat samples. The presence of high levels of *E. coli* in meat had been attributed to the contamination from faeces of infected animals or poor hygienic practices during handling, slaughtering and processing (Prince and Maalekuu, 2014).

This study has concordant results with that of a study in Nigeria when *E. coli* O157 was isolated from 2.2% of raw meat samples (Tafida et al., 2014). Diarrhea caused by enterotoxigenic *E. coli* (ETEC) is highly prevalent in young children in developing countries. Also, *E. coli* has been identified as a leading cause of food borne illness all over the world. The transport of meat from the slaughter houses is not done under refrigerated conditions and the meats are often carried under unhygienic conditions in containers or in taxis (Anihouvi et al., 2013; Fonteh et al., 2015).

The dried meats in N'Djamena and Yaoundé were exposed to direct air which carries airborne microorganisms. This explains high level of microbial contamination of dried meat (compared to the fresh meat and *Kilichi* meat). The dried meat sellers are mostly women, in Chad. Hence the high microbial load in dry meat might come from jewelries and clothing (Firew et al., 2014). Hands are also important sources when it comes to transmitting microorganisms to food. Therefore, they should always be washed before starting work, and immediately after using the toilet. The microorganisms in

kilichi can be attributed to various factors: The quality of water used during processing, the quality of seasoning ingredients (Anihouvi et al., 2013), poor school education and inexperience of the producers (Klontz et al., 1995). Also, most retailers do not protect the products from flies and expose the product to the open atmosphere. Bovine meat which is initially sterile becomes contaminated with bacterial pathogens via transmission of organisms from the exterior of the live animal, and /or from the environment.

Staphylococcus is common in unprocessed animal products and in products handled by bare hands. The high numbers of *Staphylococcus* in meat samples indicate the presence of cross contamination, which is usually related to human clothes and utensils (Firew et al., 2014). Presence of *Staphylococcus* in meat types can be worrying because *S. aureus* causes foodborne infections and diseases which range from simple abscesses to more severe toxic shock syndrome (Obeng et al., 2013). 32.5% of samples (176) were positive for *S. aureus* in cattle and pigs slaughtered in selected abattoirs in South Africa, according to Nicoline et al. (2015), which corroborates with this study. The results about total aerobic mesophilic bacteria and Enterobacteriaceae in different samples of meat were similar to the results reported by Kumar et al. (2010) for raw beef meat marketed in some parts of Tigray region (Ethiopia). The higher levels of TAM bacteria and *Enterobacteriaceae* in various types of meat (Table 1) may also be due to the fact that meat offers a rich nutrient media for microbial growth (Nfor et al., 2014).

Salmonella, a pathogenic microorganism, might have contaminated the meat as a result of poor handling by producers and/or meat sellers and contamination from the water used in washing the produce. Fresh raw meat like beef, *kilichi* and dried meat may be responsible for a number of meat borne infections and intoxications in several countries (Tidjani et al., 2013). The high level of bacterial viable counts after post washing of the carcasses in this study is in agreement with the study of Mbawala et al. (2010).

Results of API 20E strips galleries identification system revealed 51.8% of *Salmonella and* 65.2% of *E. coli.* The presence of *Salmonella* in *kilichi,* dried meat and fresh meat samples is similar to the study in Jimma town by Tasew et al. (2010) for minced meat.

Microbial harm associated with pathogenic bacteria in bovine meat products is high in Yaoundé and N'Djamena. Prolonged processing time and high temperature during processing and vending or cross contamination due to improper handling of meat or inappropriate vending practices like absence of clean water could be responsible for the high microbial counts.

Conclusion

Salmonella, E. coli, S. aureus, total areobic mesophilic

bacteria and Enterobacteriaceae were encountered in meat samples from Yaoundé and N'Djamena. The level of bacterial viable counts in this study in N'Djamena and Yaoundé is significantly very high. This study has proved that microbial adverse effect associated with pathogenic bacteria is high in meat products of these two towns and reinforces the need for those in authorities to look for means to tackle this problem. The authorities can tackle this problem by developing a management protocol control based on hygiene measures at critical points.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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