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Research Article

MICROBIOLOGICAL QUALITY OF GRILLED MEAT (SOYA) SOLD ALONG THE WAYS IN BANGANGTÉ, NDÉ DIVISION (WEST-CAMEROON)

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ABSTRACT

The aim of the present study was to assess the microbiological quality of grilled meat sold in Bangangté, West-Cameroon. Also referred to as "soya", grilled meat is a spicy popular and ready-toeat boneless beef product that is stacked and cooked by roasting with a glowing fire. As it has high nutritive values; bacteria could easily grow on it, leading to its spoilage that may be a health threat to consumers. To address the microbiological quality of the meat, a total of 96 samples were collected from 12 soya sellers. A questionnaire and observation sheets were also used to record activityrelated pieces of information. These samples underwent microbial screening according to standard protocols and specific bacteria isolates were further screened for their bio-enzymatic characteristics. Overall, 96 specimens were collected. Out of this number, 87.5% were not complaint, then unsuitable for consumption. More details indicated that 13.5% contained TAMF with loads found between 60 and 3.3×10^6 CFU/g; and that 53% were contaminated by fecal coliforms (highest value, 3.3×10⁵CFU/g). Close to 34.4% were contaminated by Staphylococci up to 3.3×10⁵CFU/g out of which 10.4% and 15.6% were positive for DNAse and coagulase, respectively. Furthermore, 86.5% of the samples were contaminated by Enterococci right to 330 CFU/g. The present work highlighted that grilled meat sold in Bangangté was contaminated with potential harmful microorganisms in connection with low hygiene. Minimal resources for investigation and local policies should be put in place in a global strategy to encourage report of cases of discomfort and address minimal quality of food used by the populations of Bangangté.

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INTRODUCTION

The general human welfare relies on healthy physical environment, food safety and type of connection amongst environmental living and non-living entities. Food safety refers to all measures designed to provide food as healthy as possible, that is, free from harmful microorganism, toxins or other derivatives. Food intoxication is therefore one of the most important concerns of all public health systems across the globe for their frequency and the burden in terms of morbidity, caretaking of affected people and its general economic impact. Deeply concerned about this issue in the 2000s, the World

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Health Organization (WHO) Member States adopted a resolution which recognized food safety as an essential aspect of public health(OMS, 2000). One of the most common vehicles of food intoxication is meat, due to richness in water and nutrients that facilitate growth of ranges of bacterial populations. Referred to as product of muscle transformation after the death of an animal (Dennaï et al., 2001), meat is free from contaminants when it is obtained from a healthy animal slaughtered and prepared in ideal hygienic conditions, but also very sensitive to microbial growth because of its high water and nutrient contents. These ideal conditions are often hardly met, however, for many populations and especially in resourcelimited communities. In fact, because, the surfaces are exposed to ambient air that provides proper conditions for microbial development (Hassouna et al., 2005), special measures should be met to preserve the product integrity as soon as it is cut. Lack or shortage of these measures is at the origin of adverse effects experienced by consumers.

In Benin (West Africa), investigations on the hygiene quality revealed that current meat production practices can lead to contamination by such harmful micro-organisms as pathogenic strains of E. coli, Salmonella enterica, Bacillus cereus, Clostridium botulinum. Clostridium perfringens, Staphylococcus aureus, Listeria monocytogenes, Mycobacterium bovis and Mycobacterium tuberculosis (Salifou et al., 2012). The type of contaminants seemed to be associated with that of the substrates. During a similar survey conducted in Dakar (Senegal) on grilled meat, it was observed that 30% of the samples were contaminated by total aerobic mesophilic bacteria, 65% by fecal coliforms, 22.5% by Staphylococci, 34% by sulphite-reducing anaerobes and 3% by Salmonella. In another one carried out on rice with fish, total aerobic mesophilic flora and total coliforms were isolated from 12.5% and 35% of samples, respectively. In that survey, no contamination by pathogenic Staphylococci, sulphite-reducing anaerobes and Salmonella was reported (Mbwala A et al., 2010). Still another one on "Kilishi" sold by street vendors in Ngaoundéré (Cameroon) resulted in the isolation beyond acceptable CFU values of Escherichia coli (30%), Bacillus cereus (23%), Staphylococcus aureus (19%), Salmonella spp. (15%), yeasts and molds (5%) (Njongmeta et al., 2004).

Hygiene rules aimed at achieving acceptable sanitation were generally not observed by those involved in meat processing of the above meat derivatives. Grilled meat is very praised by Bangangté dwellers. Cases of food-related illnesses with unknown etiologies (but likely in connection with street food) are on the rise in local health facilities. The present study aimed at addressing the microbial quality of grilled meat sold in Bangangté in order to specify the role it may play in the general health arena in the area. Basic measure to ensure food safety by vendors will also be assessed. Combined sets of results thereof will help advising ready-to-eat food item manipulators on the rules required to make their business more productive and contaminant-free in the short run. In the intermediate and long runs, the results from the present investigation will guide sensitization on food processing with regards to general and specific hygiene.

MATERIAL AND METHODS

Ethical consideration, health amenities and study population

The go-ahead was obtained from the Head of the Cliniques Universitaires des Montagnes under reference number: Ref: 2017/0019/CUM/ADM.

The present work was conducted in the town of Bangangté, the Head quarter of the NdéDivision, West-Cameroon. This division consists of four sub-divisions (Bangangté, Bazou, Bassamba and Tonga). The climate is characterized by two seasons (a dry season and a rainy season). Typically, Bangangté is a semi-urban area that hosts several incomegenerating activities conducted by a mixture of populations with different traditional and educational backgrounds, favored by the development of higher schools. One of these activities is the small business with grilled meat, a food that is particularly relished by populations of all ages.

Sample collection, bacteria detection and isolation

This descriptive study was conducted from February through May 2017. During the sample collection phase, the grilled beef (called "Soya") was bought from sellers performing by the sidewalk, in streets or on market places. All were collected in sterile plastic bags according to standard procedures and conveyed without delay to the Laboratory of Microbiology of the Université des Montagnes' Teaching Hospital for processing. All cultures, isolations and identifications were conducted according to standard protocols by the Microbiological sanitary standards of grilled meat(Société Française de Microbiologie, 2016). In short for the different bacterial species targeted, colony isolation was performed on agars that included Baird-Parker, McConkey, Plate Count, DNAse and Biles Esculine Azide (BEA) agar. In addition, a questionnaire on personal data and hygiene was filled for each participant.

Preparation of the mother solution and dilution

Meat pieces from each sample were removed from the sterile plastic bag and weighed. About 3 g from each sample was put in a labeled sterile lidded pot containing 27 mL of physiological saline. The mixture was shaken thoroughly with an electric stirrer to obtain the juice used for bacteria investigation. This solution was regarded as the mother solution to be used in the subsequent serial dilution. From this mother preparation, 2 mL were transferred into 2 mL of physiological saline (0.9% NaCl) in a test tube for the serial dilution preparation (1/2, 1/4/, 1/8, etc.). A volume of 300 μ L of the second and third dilution was thereafter, plated onto different culture agar in conveniently labelled Petri dishes (done in triplicate). These preparations were allowed to aerobic incubation 24 - 48 hours at 37 °C for total aerobic mesophilic flora and Enterococcus, 24 hours at 37°C for Staphylococcus, and 24-48 hours at 44 °C for fecal coliforms. Upon completion of incubation and in addition of the total count recorded on PCA, pink colonies on McConkey were regarded as fecal coliforms while darker ones on Baird-Parker and BEA were regarded as Staphylococcus and Enterococcus, respectively. Gram stain was carried out for all suspected colonies to attest veracity of the results. For Staphylococci, DNAse and coagulase tests were further conducted.

Coagulase and DNA setests on Staphylococci

A 24 h sub-culture on Mueller Hinton was transferred into a test tube containing 2 mL of rabbit blood plasma. This preparation was allowed to incubation at 37°C for 24 hours. Upon revelation, the coagulase-positive isolates were characterized by a coagulum (taken in mass) at the bottom of the test tube.

For DNAse test on *Staphylococcus*, bacterial colonies were streaked on fresh DNAse agar and allowed to incubate at 37°C for 24 hours. Upon completion of incubation, a 0.1 N, HCl was poured on the surface of the culture and allowed to react for 2-5 minutes. A transparent oriole that developed in the agar under the culture indicated that the strain was positive for DNAse.

RESULTS

Participants and specimens

From February 1st through May 1st 2017, 96 specimens and 12 survey sheets were collected from the 12 roasters. All were male 75% of whom belonged to the 20 through 40 years' age group while the others ages were found between 41 and 60 years. In addition, close to 58.3% never attended secondary education while 2/3 had no education at all.

Meat origin and ingredients

About 66.7% of roasters used both beef and chicken unlike the 33.3% for which beef was the only meat item for their business. For all, roasting was the key financial income-activity throughout the year. The meat used was eithers bought from the local slaughter house or from the market place. Ingredients used in this activity included onions, pepper (fresh and dry), tomatoes, magi salt, "Kankan", carrot, green pepper, garlic and oil. The dry ingredients were either kept (packaged) in lidded bottles or in plastic papers.

Hygiene of premises and equipment

Some charcoal grill consisted of drums on mobile wheelbarrows while the others were fixed, built with bricks and tiles. All were covered with a grill and equipped with a table where grilled meat was displayed and stalked. Amongst the12 sites of sample collection, 5 were carefully built and protected with tiles while the remaining ones were made of cut drums, trucks and were covered with metal gratings. The surroundings environments were unhygienic with regards to both the types and amounts of solid garbage and liquid dirty wastes that often littered the food processing environment; sometimes with onsite open gutters that attracted houseflies. Some used plank tables, slabs and trays to cut and stick the merchandise. Utensils made of plastic, metal or enamel were washed and the water used repeatedly until it was cloudily dirty. On the other hand, the dishes were washed with water and soap after each service. Knives and cutters were regularly cleaned with a piece of cloth that was often washed with soap at the end of the day for some or once every two to four days for the others. Still some cleaned their iron stall once a day while the others did it once a week. With regards to the woody cutting plank, they were rarely washed.

Personal hygiene, hygiene of operation and treatment of the unsold products

As concerned personal hygiene of the study participants, it was observed that money and food items were handled simultaneously, alongside with hand shaking with other people. All participants prepared the meat at their stalls located by the roadsides. Water was not readily available and for the little quantity they had, they usually fetched it from nearby facilities like forages (33.3%) or pipes (66.7) and used economically. All said that they washed their hands each time after toilet. Wearing of their working coat was respected by 50% of the respondents. None had received any training on food hygiene while grilled meat was never inspected by any authorities. None of the participant was observed to wash their raw meat prior to cooking and grilling, while the ready-to-eat samples were exposed to dusts and flies. All the grilled beef from production was rarely sold during the day. Unsold parts were then packed in cement paper and kept in refrigerators for the coming days after heating. Also, packaged seasoned meats were preserved 48-72 hours.

Microbiological findings

Culture and bacterial type counting revealed that the samples under study were contaminated at varying degrees with the targeted bacteria groups. Overall, twelve (12.5%) of the 96 samples were compliant to the limits at all points of view. Data related were reorganized to associate contamination rates to participants as displayed in table I.

 Table I: Level of contamination of samples for each participant

Participants	Bacterial Type (%)				
	TAMF	Fecal coliforms	Enterococci	Staphylococcus	
Participant 1	37.5	75	100	62.5	
Participant 2	25	75	100	37.5	
Participant 3	12.5	62.5	75	37.5	
Participant 4	12.5	62.5	87.5	50	
Participant 5	37	50	75	37.5	
Participant 6	23	75	100	37.5	
Participant 7	12.5	62.5	100	37.5	
Participant 8	12.5	62.5	100	37.5	
Participant 9	12.5	25	62.5	25	
Participant 10	37.5	75	100	50	
Participant 11	0	50	62.5	0	
Participant 12	0	0	62.5	12.5	

TAMF: Total aerobic mesophilic flora

All the bacteria types that represent hygiene level indicators were present in the samples. The most frequently contaminated specimens were collected from participant 1 and the least from participant 12. In addition, *Enterococci* were detected in sample from all participants. The overall percentages of contamination per bacteria type were summarized as shown in Table II.

Table II: Frequency of contamination

	Bacterial type				
	TAMF	Fecal coliforms	Enterococcus	Staphylococcus	
Frequency of contaminated	13	53	84	33	
Percentage	13.5	55.2	87.5	34.4	

Data analysis revealed varied contamination rates for the four microbial categories targeted by the survey. Special notice was that some samples were contaminated with more than one microbial types and that *Enterococci* were detected in sample from all participants. Further characterizing *Staphylococcus* isolates indicated positive DNAse tests on close to 10.4%, while coagulase positivity was recorded in 15.6%.

DISCUSSION

The present survey aimed at assessing the microbiological quality of grilled meat produced and consumed in Bangangté. The primary findings that focused on hygiene and sanitation in the processing units revealed that the risk for contamination was high with regards to the working environments, care of the material used and the water scarcity that was reported in previous studies(Kouamouo *et al.*, 2013), amongst others. Moreover, the fact that the participants' level of education was low might further be regarded as a helpful clue that could justify the poor hygiene observed.

In these regards, most stalks were poorly cleaned while the grill meat were not protected from dust and flies that are recognized as potent engines (vectors and vehicles) for contamination by microorganisms in general. In fact, the poor hygienic practices substantiated in the present survey by poor water used in cleaning, poorly washed utensils, dirty hands, unclean dresses, handling of money/ soya simultaneously and hand shaking with visitors that was observed during this investigation could further explain the high contamination rates recorded. In these working conditions, no comprehensive list of possible enabling factors could actually be made in fact; but indicators like Enterococcus and fecal coliforms could safely and reliably serve, as indicated in the above results, and consistent with previous reports (Edema et al., 2008). Similar findings were also reported by other authors who worked on grilled sheep meat in Cotonou, Benin (Hippolyte et al., 2007). All participants in the present work fetched water from nearby water sources: 33.3 % from forages and 66.7% from the national company in charge of water distribution, contrary to the report from Hippolyte et al.(2007) in which 87% of the roasters used water from wells and 13% from pipes (Hippolyte et al., 2007). What could be expected from the results of the present survey was a lower rate of contamination with the availability of pipe-borne water for more than 2/3 of the participants and heating that would be effective in getting rid of several groups of microbes. Sources of contamination of the final meat product could, in fact, have several origins including:1- the health status of the animal as it might be infected, becoming thereby the source of a zoonotic episode; 2the point of sale that is either at the level of the raw meat sellers on the market place or at the slaughter house where the meat can be contaminated by the contents of the animal gut or by microorganisms from the surrounding environment; 3- during transportation to the roasting site; 4-during manipulations by the roasters. At the latest level however, post-roasting contamination is the most likely possibility with regards to the conducive working environments, consistent with Edema et al. (2008). Heating during roasting would actually reduce microbial loads and make the soya safer for consumers. More on the protection/preservation of the final attention paid product would virtually improve the compliance rates as

already pointed out in other surveys (Mbwala *et al.*,2010). If for instance, all vendors confessed that they regularly washed their hands after they have used the toilets; what they said was not consistent with the detection of fecal coliforms and *Enterococcus*, which are key indicators of contamination from fecal origin.

About the microbiological quality of grilled meat and as stated earlier, the present investigation revealed that it was poor in general. Bacteria could easily be transmitted by all the above means, as conducive engines are frequently present from one end to the other throughout the production chain with key bacteria types frequently detected from the large majority of the samples.

The total aerobic mesophilic flora (TAMF) represents hygiene test bacteria. Their count remains the key to appreciate the general microbiological quality of samples and the level of hygiene practices observance in food production and processing units. If regarded as the single indicator of contamination, the rates of their detection (13.5%) would be relatively satisfactory. Similar findings (≈12.5%) were reported in Dakar Senegal when rice with fish was analyzed; significantly lower than those obtained from grilled meat (30%) (Mbwala et al., 2010). Members of this groups included fecal coliforms like Enterobacter spp., Citrobacter spp., Klebsiela spp. and Escherichia coli (Hippolyte et al., 2007). Overall, coliforms were detected in 55.2% of specimens in the present survey. This rate was almost similar to the one reported in Senegal (65%), but different than that recorded when rice with fish was analyzed (35%) (Mbwala Aet al., 2010).

Staphylococcus was isolated from 34.37% of specimens, almost similar to the findings from the study on grilled meat in which the contamination rate was high (Mbwala et al., 2010). Unlike coliforms, Staphylococcus are more ubiquitous, therefore less reliable indicators of food contamination. This characteristic is favored by their higher ability to resist and perpetuate in stressful environments including air, water and the soil(Goudiabyet al., 1989). This assumption is consistent with the frequency of DNAse and coagulase positivity that characterized pathogenic strains (10.4% and 15.6% of the isolates in this survey). These pathogenic strains, however, are recognized as potential etiologies of several human disorders throughout the world (Bourgeois et al., 1996). Their real impact and that of the others (undetected etiologies) in the study community is yet to be addressed. It would be necessary to know how many consumers actually develop food intoxication upon meat ingestion. Present data do, therefore, not provide such opportunity, but specific strategies could be put in place by researchers and the Urban Council to encourage reporting discomforts after meal in general. This is crucial in such a fastgrowing community like that of Bangangté where more and more people are used to fast-food.

The extreme rates of contamination were recorded with *Enterococcus*, detected in 86.5% of the specimens under study out of the overall 87.5% non-compliance rates. Otherwise, only 1% would globally be imputed to the use of other indicators in the meat quality assessment. Together with fecal coliforms as stated earlier in the present development, *Enterococci* are stronger indicators of fecal contamination, consistent with the above conclusions and the role of basic hygiene practices in the

roasting business. According to the current standards, a single cell/colony is enough to qualify a food as not proper for consumption. Regards to these two groups could primarily, but sustainably be used in a context of resource limitation for food quality assessment. From the above findings it would not be exaggerating to anticipate that discomfort due to food poisoning could be common in Bangangté; but the amplitude and the economic burden would also be difficult to estimate because the cases are not reported. Paying attention to food quality could, however, be a solid opportunity to improve the overall economic performance for the whole country. For instance, Kien et al. (2017) estimated that in Vietnam, the average costs per treatment episode and per hospitalization day for food borne diarrhea case were US\$ 106.9 and US\$ 33.6. respectively; with the indirect cost (time loss to patient, their relatives due to the patient's condition) representing the largest part (51.3%). These authors further observed that direct medical costs accounted for 33.8%; while direct non-medical costs (patient and their relatives) represented 14.9% (thought cost levels and compositions varied by level of health facilities). In the United Kingdom, it was found that foodrelated health conditions were responsible for about 10% of morbidity and mortality, costing about £6 billion annually (Rayner et al., 2005). Such high costs were also reported from Hungary (Vajda et al., 2016). They are most likely higher but under estimated in middle- and low-income countries like Cameroon, due to shortage of financial and human resources. Taking into account these aspects would virtually not only impact the consumer, but also the sustainability of the activity for the roaster who, for most, perform it as the main financial resource for family incomes in and environment where others restauration units would provide better food quality to their clients and force them out of business.

Accordingly, couldn't *Enterococci* be the most targeted in such instances? It is clear that some researches are not conducted because of limited financial resources. More works would be necessary before appropriate answer is provided. Why the rate of contamination by TAMF is lower than that of *Staphylococci* and fecal coliform resist clear elucidation, but subtle culture media and stochastic micro-environment properties might play key roles in bacteria fitness, survival and growth. For instance, the chemical composition of ingredients that is likely to vary from one respondent to the other remained unknown and could influence bacteria growth by altering the chemical properties of the culture media.

CONCLUSION

The rate of contamination was very high, most likely in connection with poor hygiene and sanitation. Report of related disease conditions are not made; but appeared crucial in assessing their likely high burden that is supported by the national economy and in preserving the sustainability of the activity for the roasters. Minimal resources for investigation and local policies should be enforced in a global strategy to encourage report of cases of discomfort and address minimal quality of street' food in Bangangté.

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