

Sanitary Risk Factors and Microbial Contamination of Grilled Meats Sold in Cotonou, Benin

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Abstract Grilled meat is a rich foodstuff in protein and very important for human consumption. Its quality is all the characteristics that give it its organoleptic, nutritional, hygienic and technological properties. The aim of this work was to evaluate the sanitary risk and the microbial contamination of grilled meat sold in Cotonou, Benin. To reach our goal, a semi-structured investigation was conducted in the meat grilling production shops focusing on the characteristics of the company, the method of preparation of the meat, the type of meat produced and methods of preserving meat. A total of 50 samples of meats samples were analyzed for fecal coliforms, *Escherichia coli*, *Staphylococcus spp.*, sulphite reducing anaerobes and total aerobic mesophilic counts. The susceptibility to conventional antibiotic was investigated by the disk diffusion method. The inspection of the meat grilling enterprises revealed that 73% of these companies are located on the edge of the high-traffic public road, 85% of which is sold by a person and 15% by 2 people. This activity is conducted solely by men (100%) in Cotonou and dominated by Nigerians (57%). Most of the grilled meats sell meat from both chickens and mutton (82%) and the majority (70%) get their supplies from slaughterhouses market. Before grilling operations, 97% of the sellers make a general cleaning and in case of slump 80% use the refrigeration to preserve their product. Total aerobic mesophilic flora, *Staphylococcus*, coliforms and *Escherichia coli* load recorded with chicken samples are higher than those observed on mutton samples are. Considering grilled mutton, *Staphylococcus* load (6.9×10^3 CFU/g) is higher than that of *E. coli*'s (1.4×10^2 CFU/g). The antibiogram test on strains of *Staphylococcus* to reveal overall that the resistance rates of *Staphylococcus* strains to antibiotics are less than or equal to 50% regardless of the antibiotic with a very strong inhibitory activity (98% inhibition) vancomycin and ciprofloxacin.

Keywords: grilling, risk factors, meats, microbiological quality, BENIN

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1. Introduction

Food safety remains a global concern despite efforts made by health and hygiene organizations [1]. Food contaminations occupy a prominent place in conditions closely related to global mortality rates. The estimated number of deaths is more than 2 million per year, and mainly children, only through the consumption of food and water, of inadequate hygienic and sanitary quality.

Food contamination therefore remains a real and permanent risk, especially in cities where this risk is closely linked to the consumption of street food [1,2]. Street food is characteristic of developing countries such as Benin [3]. It enables more than 80% of urban populations to eat easily and at a lower cost [4].

An excellent source of animal protein and important for human consumption, grilled meat called "Tchintchinga" is included in this class of street foods. It is the product of transformation of the muscle after the death of the animal [5] and its quality is the set of characteristics that give its

organoleptic, nutritional, hygienic and technological properties. It is often preferred in roasted form, which is classified among the main traditional methods to limit losses. Nevertheless, this feeding habit is a potential reservoir of consumer contamination by microorganisms [6]. Slaughterhouses are one of the major critical points of meat hygiene. Thus, 80 to 90% of the microflora found in meat comes from slaughterhouses [7]. Personnel, tools and surfaces contribute to carcass contamination, especially if they are poorly maintained. Thus, unhygienic handling during slaughter, carcass preparation and processing can lead to very large surface contamination that can affect consumer health and meat quality (organoleptic alteration).

The organisms involved in food poisoning are numerous and the most common are *Salmonella*, *Escherichia coli* and *Staphylococcus aureus* [3,8,9]. Ingestion of bacteria or toxins could cause dysfunction of the consumer's body causing digestive infections [10]. Vectors of those intoxications are food products such as meat (cattle, poultry, swine and goats), dairy products (cream, cheese and custard) and raw vegetables [11]. Symptoms usually include vomiting, diarrhea, and severe abdominal pain in men. This contamination is also responsible for death in young animals on the farm [12,13]. Bacteria involved in collective food poisoning are frequently isolated from the manufacturing processing, transportation and sales environment [9,14,15,16]. Preventive treatment is neglected in favor of the curative [17].

In Cotonou, there is a multiplication of meats grilled business known mainly called "Tchintchinga". In these meat-grilling shops, meat undergoes several manipulations before being delivered for human consumption. The aim of this study was to evaluate the sanitary quality of the meat grills in the city of Cotonou, Benin.

2. Material and Methods

2.1. Sample Size and Kind of Meat Product

For the investigation, the sample size is calculated inspired from the work of Kadam and Bhalerao [18]. Thus, the formula use was $n = p (1-p) / [e / 1.96]^2$ (n = minimum sample size required; p = estimation of the expected proportion; e = tolerated margin of error (%) in the study=5%). In our work, 100 vendors were selected using the "convenience" sampling technique [19] for the investigation. During the investigation, steakhouses with both chicken and mutton meat were selected for the collection of samples that will be analyzed in the laboratory. For this study, 50 vendors were selected for the samples collections.

2.2. Investigation

A semi-structured survey using a questionnaire was carried out from 7 pm with the meat vendors [20]. This questionnaire took into account the type of customer, the type of meat, the safety-related to the selling environment and probable cases borne outbreaks associated and other parameters such as the vendor's age, type of meat, and form of cooking, selling place and packaging type.

2.3. Samples Collection

Two kind of ready-to-eat meat (mutton and chicken) sold braised on a rack were collected for microbiological analysis. For each kind of meat, 50 samples were selected. Thus, 100 samples were collected for microbial analysis. The samples were collected in sterile Stomacher papers then transported to laboratory in icebox (4-8 °C).

2.4. Microbial Analysis

For the microbiological analysis, 10 g of each meat sample was added to 90 ml of sterile bacteriological peptone (Oxoid, Hampshire, England) and then was incubated at 37 °C for 1 to 3 h the enrichment [21]. Mesophilic Aerobic Flora (MAF), staphylococci, fecal coliforms (FC) and total coliform (CT) strains, *Escherichia coli* and *sulfate-reducing bacteria* (SRB) were enumerated. From the incubated suspension, a decimal dilution was made with peptone water (BioRad, Paris, France). Each dilution (0.1 ml) was spread on Baird Parker agar (Biokar Diagnostics, France) with egg yolk [22-23] before it incubation at 37 °C for 48 hours for gram positive cocci's. For *Enterobacteriaceae*, EMB agar (Biokar Diagnostics, France) was used to seed the inoculum and incubated at 37 °C for 24 h. Trypticase-Sulfite-Neomycin Agar (TSN) was used to identified *sulfate-reducing bacteria*.

The contamination frequency was calculated from the ratio of contaminated products on all the products whereas the prevalence was obtained by the ratio of the strains isolated on all the biological products tested. Once isolated, the microorganisms were identified using classical morphological (gram staining, shape of bacteria, and Ziehl-Neelsen staining) and biochemical characters (sugar fermentation, Oxidase, Methyl red-Voges-Proskauer, indole and Catalase test) related to the genus identification techniques [24,25].

2.5. Susceptibility of *Staphylococcus* Isolates to Seven Antibiotics

As the *Staphylococcus* strains are the major pathogen isolated from ready to eat meat product [8], the susceptibility of the identified *Staphylococcus* to seven conventional antibiotics, provide by Oxoid (England) was performed using EUCAST recommendations and interpretation [26]. The tested antibiotics (Bio Mérieux, France) were ciprofloxacin (CIP 5 µg), penicillin G (P 10 µg), gentamicin (GM 10 µg), oxacillin (OX 5 µg), trimethoprim sulfonamide (SXT 1.25/23.75 µg), vancomycin (VA 30 µg) and oxy-tetracycline (OT 30 µg).

2.6. Data Analysis

The results of the survey were analyzed with the SPSS 19 (Chicago, USA). Descriptive statistics such as prevalence, mean and standard deviation were calculated for the quantitative variables. A significant difference between the mean was determined. The mean probability of ready-to-eat meat contaminated at the level posing a risk to human health was calculated as the proportion of meat multiplied by the proportion of sold meat. The main parameter used

were the age and nationalities of vendors, the type of meat, the form of cooking, selling place, the packaging type and the hygienic precautions. The Graph Pad Prism 7.00 software was used for the realization of graphs. The threshold of statistical significance was set at $p < 0.05$.

3. Results

3.1. Identification of the Different Potential Sources of Contamination of Grilled Meats

3.1.1. Sales Environment of Tchintchinga

Tchintchinga is sold directly at the cooking place during the grilling time on grid (Figure 1). The interior of this device receive the charcoal that provide the fire. The grilled meat are often sold in recycled paper, or with

cement or in dishes. The dishes are observed into shops located at the refreshment area.

3.1.2. Characteristics of Grilled Meat Sales Shops

Our survey shows that grilled meat shops of Cotonou do not have a name and only men carry out this activity. These men are mostly coming from Nigeria (57%) and Niger (40%). In addition, it has been found that Beninese and Guineans are also present in this activity (Figure 2).

The age range of men in this sector ranges from 25 to 65 years old. Nevertheless, we note that the number decreases when the age increases especially from 50 years (Figure 3).

Most (73%) of grilling shops are located on public highways followed by those located near the bush restaurants (19%) and near refreshment bars (8%). In addition, it was reported that the activities of these processing units start in the afternoon (between 4 pm and 5 pm).



Figure 1. Picture showing the Tchintchinga sales environment in Cotonou

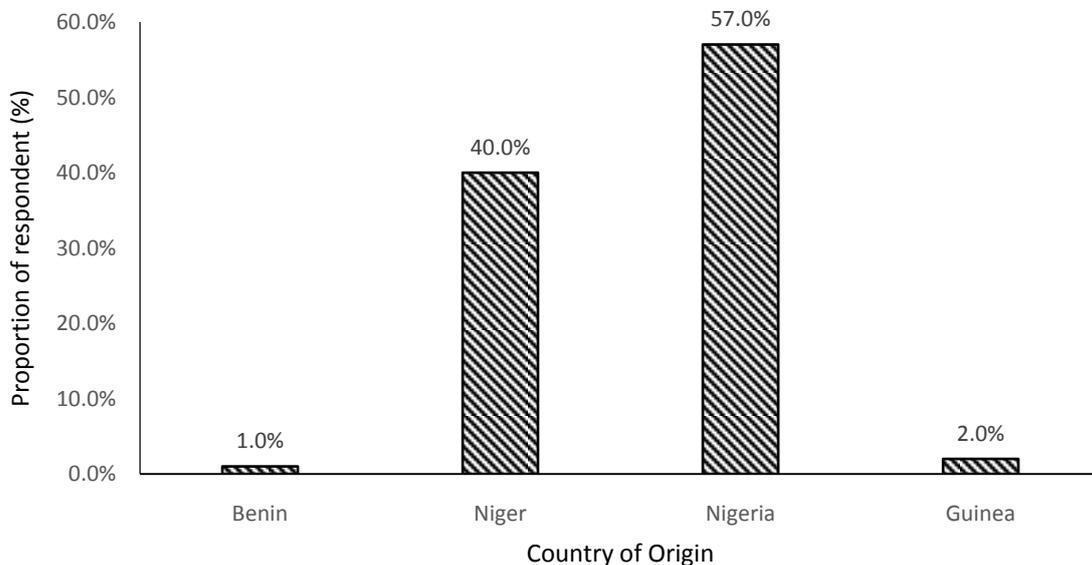


Figure 2. Country of origin of grilled meat sellers

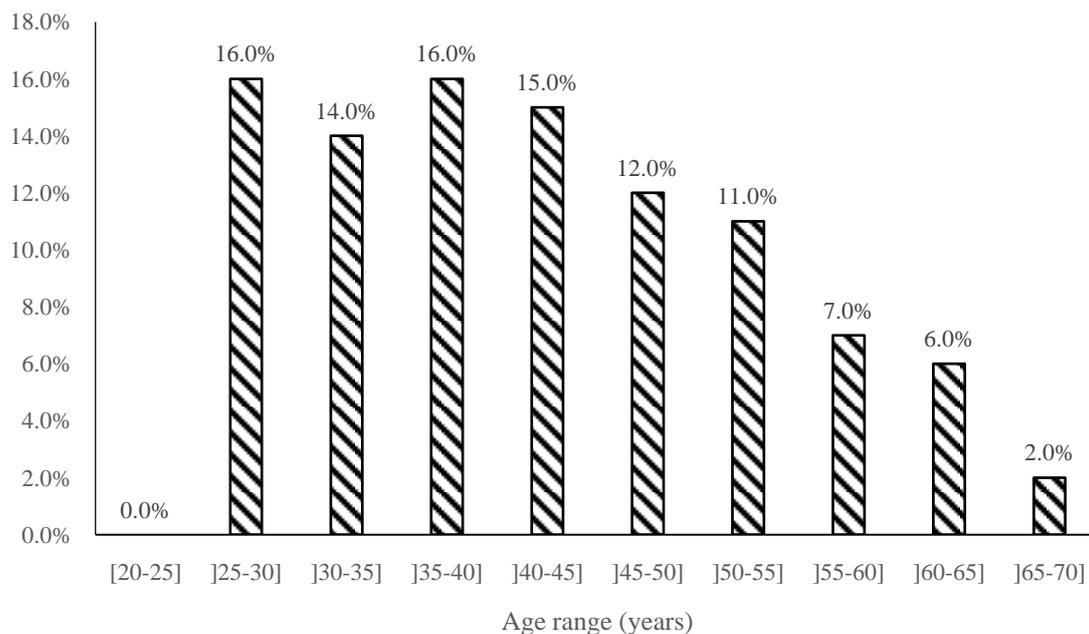


Figure 3. Distribution of grilled-meat vendors surveyed in Cotonou according to their age

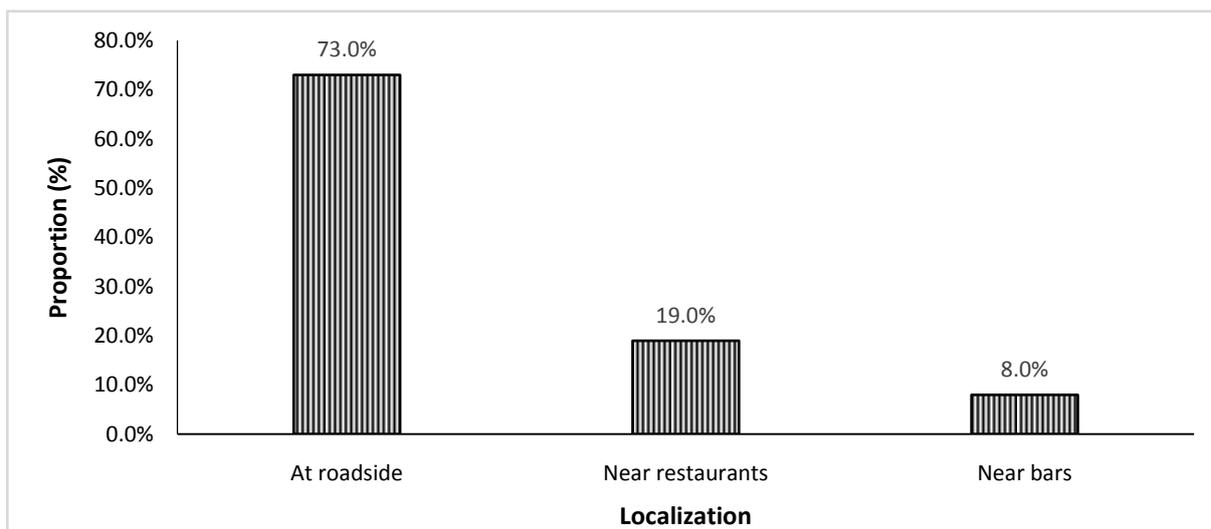


Figure 4. Repartition of grilled-meats shops in Cotonou

3.1.3. Acquisition and Transport of Meat for Grill

Tchintchinga are mainly (82%) composed of mutton, associated or not with chicken and / or beef meats (Figure 4). Supplies are made at the Zongo market (70%) and Akpakpa slaughterhouse (30%) for mutton and beef and in fishmongers for chicken meat. Once acquired, meats are often transported with motorcycles to their place of transformation.

3.1.4. Sanitary Arrangements Made by Sellers at Their Point of Sale

Daily, before grilling operations, 97% of vendors surveyed admitted to cleaning their displays whereas 1% declare not take any precaution. In case of non-exhaustion of stock, two methods of conservation were mentioned (Figure 6). It appears that refrigerated storage was the most common (80%) followed by no disposition (11%) and freezing (9%).

3.2. The Main Pathogens Isolated in Grilled Meats

The results of the microbiological analysis show that 100% of roast mutton collected in Cotonou is of unsatisfactory hygienic quality considering total aerobic mesophilic flora, *Staphylococcus*, coliforms and *Escherichia coli* (Table 1). It is observed that these meats (mutton and chicken) are free of *sulfate-reducing bacteria* and therefore of satisfactory hygienic quality for this germ. Globally, independently to the bacteria (Total aerobic mesophilic flora, *Staphylococcus*, coliforms and *Escherichia coli*) the load recorded with chicken samples are higher than those observed on mutton samples for *Staphylococcus* ($6.9.10^3$ CFU/g) found on grilled mutton is higher than that of *Escherichia coli* ($1.4.10^2$ CFU/g). Nevertheless, the chicken grille's *Staphylococcus* load (1.10^4 CFU /g) is lower than that of *Escherichia coli* ($5.4.10^2$ CFU /g).

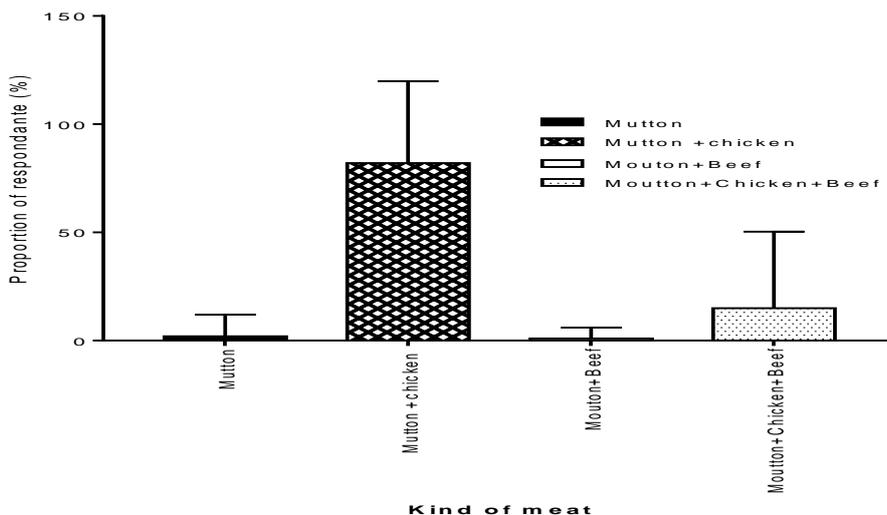


Figure 5. Types of meat found on the shelves of Tchintchinga sellers in Cotonou

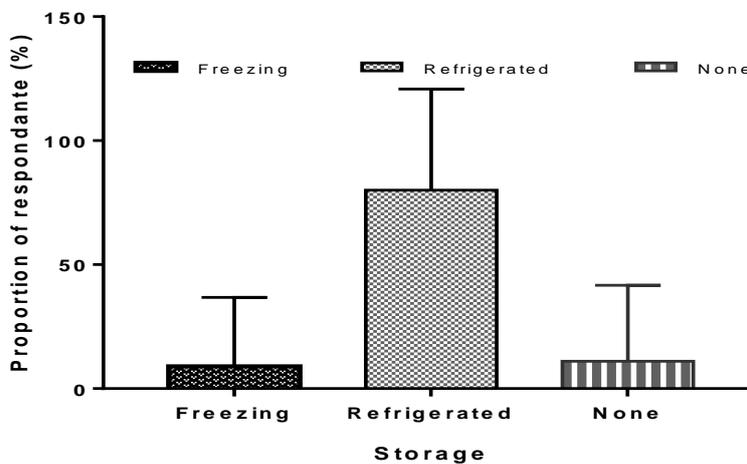


Figure 6. Treatments of the rest of Tchintchinga in case of slump the day before

Table 1. Average number of microbial load per Germ on grilled meat in Colony Forming Units per Gram (CFU/g)

	Mutton	Chicken
Total aerobic mesophilic flora	7.5*10 ⁶ CFU / g	7.7*10 ⁶ CFU / g
<i>Staphylococcus</i> spp	2.4*10 ⁴ CFU / g	5.9*10 ⁴ CFU / g
Coliforms	9*10 ³ CFU / g	1.9*10 ⁴ CFU / g
<i>Escherichia coli</i>	1.4*10 ⁴ CFU / g	4.9*10 ⁴ CFU / g
<i>Sulfate-reducing bacteria</i>	0 CFU / g	0 CFU / g

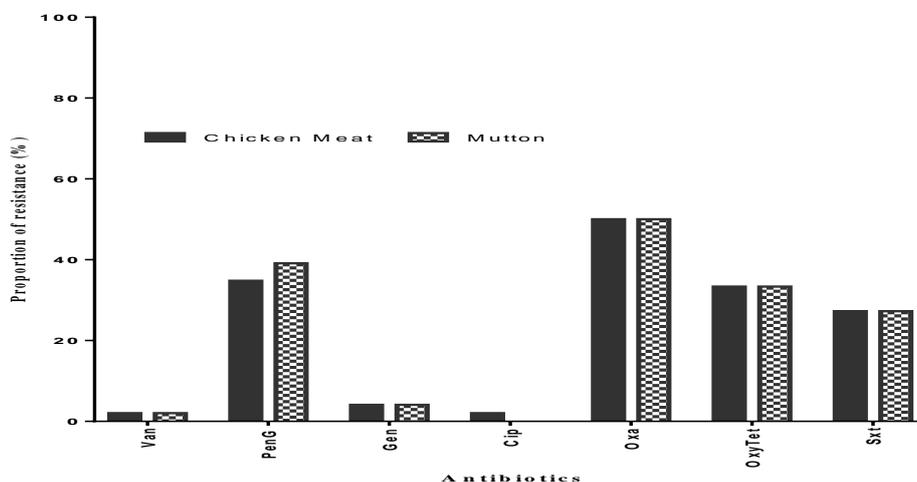


Figure 7. Resistance profile of Staphylococcus strains isolated from Tchintchinga sold in Cotonou

3.2. Antibiotic Susceptibility of *Staphylococci* Strains Isolated from Tchintchinga Samples

The susceptibility of *Staphylococcus* strains to the seven antibiotics tested is variable ($p < 0.0001$). Overall, these resistance levels are less than or equal to 50% regardless of the antibiotic. The type of meat from which the strains were isolated has no effect on *Staphylococcus* resistance ($p = 0.9231$). Very strong inhibitory activity (98% inhibition) of vancomycin and ciprofloxacin was observed independently to the origin of the strains. However, high resistance is observed with oxacillin (50%), penicillin G (36%) and oxy-tetracycline (33%).

4. Discussion

4.1. Assessment of the Results of the Investigation

The evaluation of the sanitary quality of the meat dishes in Cotonou allowed us to assess their level of microbiological quality and the general hygiene level of the grill production technique. In our survey, men (100%) dominate the meat-grilling sector in Cotonou and the majority of these men are from Nigeria (57%) with an age range of 25 to 65 years. Compared to a similar study conducted in Senegal, Wade et al. [27] reported different results. Thus, those reported that 97.5% of persons involved in the activity are men, and 47% Nigerian. In addition, those authors founded that an age group varying from 15 to 50 years and over in Dakar, Senegal. The study environment (locality or country) could explain this difference.

Places of preparation and sale of street foods have been reported to be potential sources of contamination of these foods. In our case, these places of preparation are often located next to the gutters at the roadsides. Indeed, the results of our investigation revealed that 73% of grilling shops are located on the edge of public roads. At these places the grilled-meat are exposed to many sources of contaminations. This study confirms the results of Curtis and Cairncross [28] and Obrist et al. [29] who reported that street-food preparation sites are potential sources of their contamination. Considering this affirmation, their preparation and selling environment may cause the probable sources of food poisoning inducing diarrheal diseases and pathologies.

Our study reveals that 82% of grilled meat sellers use mutton and chicken meat compared to 18% who use chicken and beef meats. Most of these sellers are sourced from a fixed source (70% at Zongo market and 30% in the Cotonou slaughterhouse) for mutton and beef but variable chicken meat (in different fishmongers). The contamination of grills by the source of supply is a very important parameter for the evaluation of the microbiological quality of grilled meats. After fresh meat supply, the major (97%) part of the investigated population reported doing general cleaning displays. Our found in this study is different from

the similar study stating that 98% of meat grilling sellers do not follow good hygiene practices [30].

It should also be noted that 80% of the sellers surveyed keep their product in refrigeration, 9% in freezing and 11% who make no provision. This result is different from that of Wade [27] according to which 53% stock their product in freezing and 43% take no precaution. This difference could be explained by a lack of means for sellers to afford a freezer or ignorance of good hygiene practices to respect. However, according to Afssa [31], the non-conservation of meat by some sellers would favor bacterial multiplication, because storage in the freezer stops microbial growth. Compliance with good hygiene practices and the storage or preservation of grilled meat in the freezer helps to ensure the quality of the product and thus guarantee the safety of the consumer's health.

4.2. Assessment of the Microbiological Quality of Grilled Mutton and Chicken Meat Sold in Cotonou.

Our data shows that a flora in *Staphylococcus*, *E. coli*, coliforms, aerobic total mesophilic flora, and *sulfate-reducing bacteria*, characterizes the samples of chicken and mutton. These same germs are among those revealed by the work of Salifou et al. [5]. Also, note that the bacterial load, per gram of grilling, of *Staphylococcus* is higher than that of *E. coli*'s. This observation corroborate those of Salifou et al. [5] in their study on the factors that influent the characteristics and quality of meat. This bacterial contamination comes from non-compliance with hygiene measures. When exposed to the open air, the meat could be contaminated by the customer who often handles it with bare hands and the environment (air, flies, insects, etc.). In addition, sellers of tchintchinga should have a good working method; there should be a good distribution of tasks. For example, a person must be in charge of money collection and another responsible for the sale. This accumulation of task would promote microbial contamination of the meat. This high bacterial contamination of grilled meat observed in our study could be because the meat is not well cooked, because the cooking of the food allows a strong reduction of the microbial load if the heart temperature of the food is high. The rare cooking of meat is not sufficient to provide protection in the event of contamination by a pathogen.

4.3. Susceptibility of *Staphylococcal* Strains of Grilled Mutton and Chicken

The inhibition test revealed that the *Staphylococcus* strains were resistant to oxacillin (50%); penicillin G (36%) and oxytetracycline (33%), however, a very strong inhibitory activity (98%) of vancomycin and ciprofloxacin was observed, regardless of the type of meat. Similar result was reported in studies conducted in Ivory Coast [32] and Benin [33]. These differences in antibiotic efficacy on *Staphylococcus* strains are due to a difference in microbial load per gram of meat.

5. Conclusion

This study provide highlight the probable origin of the contamination of grilled mutton and chicken meat by observing hygiene practices. We estimate the consumption risk of the meat produced in ready-to-eat steakhouses. Thus, good transport practices for meat and grilled mutton and chicken meat must be improved. Microbiologically, staphylococci strains are most isolated in grilled meat samples. In addition, strong resistance of staphylococcal strains to certain molecules is observed. Nevertheless, good inhibitory activities of some reference molecules are observed and can be advised for the treatment of staphylococcal infections.

Conflicts of Interest

The authors declare no conflict of interest.

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