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Assessment of Health Risks Related to the Consumption of Minced Meat Sandwich

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Abstract

The objective of this work was to assess the health risks associated with the consumption of minced meat sandwiches, sold in the informal sector in Brazzaville in the Republic of Congo. A survey on the application of hygiene rules was conducted in parallel with a bacteriological analysis of cooked minced meat. The enterobacteria isolated from this food were identified and antibiotic resistance testing was performed. The investigation revealed shortcomings in respect of basic hygiene rules, and 56% of the sandwiches analyzed were of bacteriological quality unsatisfactory. The non-compliance of the sandwiches was caused mainly by the presence total aerobic mesophilic flora (71.43%) and total coliforms (57.14%). In contrast, not all samples were contaminated with anaerobes sulfito-reducting bacteria and *Salmonella*. Five species of *Enterobacteriaceae* were identified: *Escherichia coli* (35.30%), *Proteus vulgaris* (11.76%), *Klebsiella oxytoca* (11.76%), *Citrobacter* spp. (23.53%) and *Enterobacter cloacae* (17.65%). Of these, 42.65% were resistant to 75% of antibiotics tested: Cefalexin (17.24%), Ceftriaxone (48.28%) and Norfloxacin (34.48%). In contrast, all strains were sensitive to Nitrofurantoin. Minced meat sandwiches sold in informal sector in Brazzaville can be source of enteropathogens, susceptible to expose consumers to foods poisonings.

Keywords: Pathogenic bacteria, Food contamination, Health risks, Minced meat, Brazzaville

1. Introduction

The minced meat sandwich is a ready-to-eat food consisting of cooked minced meat sandwiched in bread. It is widely consumed in Brazzaville. Meat is considered as food of choice because of its nutritional value. However, for the same reason, it is a favorable environment for bacterial proliferation (Phillips et al., 2001). Bacterial activities have a great influence on the organoleptic and hygienic quality of food products (Guiraud, 2003). Indeed, they can cause changes in taste, color, smell and produce metabolites harmful to the health of consumers. In the case of meat, the harmful effects of the bacterial flora are favored by the chopping operation it undergoes (Phillips et al., 2001); the minced meat is so a fragile food which must be strictly monitored because of the danger due to these alterations and the presence of potentially pathogenic agents.

Meat foods ready to eat have been repeatedly criminalized in collective food poisoning throughout the world (Cohen & Karib, 2006); sandwiches and salads have been implicated in food borne epidemics (Christison et al., 2008). These foods are often prepared by hand; this direct contact can lead to an increased incidence of contamination by pathogens such as

Staphylococcus aureus (Kotzekidou, 2013). Handling, storage conditions, and food exposure at the time of sale are factors that influence the bacteriological burden of ready-to-eat foods.

In the Republic of Congo, minced meat sandwich is generally sold in the informal sector at the level of mini-feeds. In these outlets, often poorly designed (one room with only one door serving as an exit and entrance), the preparation and sale of sandwiches are done in parallel with the sale of other merchandise merchandise in the same premises. This situation does not guarantee the bacteriological quality of this food, which represents a risk to the health of consumers. In addition, despite the increased incidence of food-borne illnesses, minced meat used in these fast food establishments has never been microbiologically investigated.

This work consists of an evaluation of compliance with hygiene rules at the point of sale, the bacteriological quality of minced meat and the antimicrobial resistance of enterobacteria, possibly present in this foodstuff.

2. Material and Methods

2.1 Investigation

The survey was conducted in the form of an interview and observation of staff at work. It involved seven sellers at five points of sale (A, B, C, D and E respectively), three of which were at point of sale B. Sites A, B and C are located in district 2 Bacongo, while sites D and E are located in district 1 Makélékélé. The main selection criteria were: own the store and be willing. The choice of points of sale was made taking into account the size of the influx of customers. The survey mainly consisted in evaluating the application of good hygiene, preparation and sales practices, but also the profile of the sellers.

2.2 Sampling

A total of twenty-five (25) ground meat sandwiches were collected, at the rate of one (1) sandwich per visit and five sandwiches per points of sale (Table 1). The samples were packaged in sterile plastic bags and transported immediately to at laboratory in cooler.

Table 1. Provenances and codification of samples

Borough	Point of sale	Number sellers	Number samples
Bacongo	A	03	5 : B_1, B_2, B_3, B_4 et B_5
Bacongo	B	01	5 : S_1, S_2, S_3, S_4 et S_5
Bacongo	C	01	5 : C_1, C_2, C_3, C_4 et C_5
Bacongo	D	01	5 : L_1, L_2, L_3, L_4 et L_5
Makélékélé	E	01	5 : F_1, F_2, F_3, F_4 et F_5
Total		07	25

2.3 Bacteriological Analysis

Bacteriologicals analyses were performed according to the standards techniques reported by Joffin and Joffin (2003). They included enumerations of the total aerobic mesophilic flora at

30°C (FMAT), total coliform (CT), fecal coliform (CF), anaerobes sulfito-reducting (ASR) and *Staphylococcus* suspected pathogens (SSP) and a search for *Salmonella*.

2.3.1 Sample Processing

About 25g of minced meat was homogenized in 225 mL peptone water and mixed properly. The homogenate was then filtered using a filter paper. The filtrate obtained constitutes the prime solution (dilution 10^{-1}) from where decimal dilutions have been made.

2.3.2 Enumeration and Research of Different Flora

- *Enumeration of the FMAT*. One (1) ml of the 10^{-11} and 10^{-12} decimal dilutions were respectively inoculated deep down in PCA supercooling agar. After solidification, 4 mL of agar was surface-cast to obtain a double layer. Incubation was performed at 30°C for 72 h (AFNOR, 1999).

- *Enumeration of coliforms*. Total and fecal coliform enumeration was performed by inoculating 1 ml (10^{-8} and 10^{-9} dilutions) on deoxycholate agar. Incubation was for 24 hours at 37 °C and 44°C, respectively (ISO7251, 2005).

- *Enumeration and search for SSP.* It was carried out by spreading 0.1 ml (10^{-4} and 10^{-5} dilutions) on the surface of Baird Parker agar. Incubation was performed at 37°C for 48 h (ISO 6888-1, 1999). The coagulase test was performed by mixing a suspicious colony in 5 mL of heart-brain broth, followed by incubation at 37°C for 24 hours. A volume of 0.3 mL of rabbit plasma was then mixed with 0.1 mL of the culture obtained and then incubated at 37°C for 24 hours. Coagulation of more than half the volume means a positive result.

- *Enumeration and SRA*. 1 ml of the stock solution was seeded into Tryptone Sulfite Neomycin (TSN) agar. Incubation was done for 24 h at 37°C (ISO7937: 2005).

- *Salmonella* search

Pre-enrichment was performed by incubating the stock solution at 37°C for 18h. Then 0.1 ml of the pre-enriched solution was transferred to 10 ml of selenite cystine broth contained in a test tube. After homogenization, the tube is incubated at 37°C for 24 h. Isolation was achieved by spreading approximately 50µl of enriched medium on the surface of Salmonella-Shigella agar before being incubated at 37°C for 24 h. Finally, the identification was done using the gallery API 20E as described by the manufacturer.

2.4 Results Interpretation

Only the Petri dishes containing between 15 and 300 colonies were considered. The results have been interpreted on the basis of criteria defined by the ministerial decree of December 21, 1979 revised on April 27, 2006 (French Republic, 2006).

2.5 Identification of Isolated Enterobacteriaceae

After enumeration, twenty colonies were randomly selected for biochemical analyzes. The isolates were purified by repeated subculturing, then store at 4°C on sloping agar. The following characters were determined: spore production, Gram type, motility and production of catalase; the suspected strains were identified using the integrated Enterobatteri system (Ref. 71714) as described by the manufacturer.

2.6 Antibiotic Resistance Testing

About 5 ml of nutrient broth was inoculated with a colony of the test strain and incubated at

37 °C overnight at an inoculum density equivalent to a 0.5 McFarland turbidity standard. A sterile cotton swab was moistened with each isolate and used to swab duplicate Muller-Hinton agar plates. Then, the plates were left to dry for about 5 min before placing the antibiotics discs on each of the plates as described by Bauer, (1996). Antibiotic discs and concentrations used were: Ceftriaxone (CRO, 30µg), Norfloxacin (NOR, 10µg), Cefalexin (CN, 30µg) and Nitrofurantoin (FT, 300µg). The diameter of the inhibition zone has been measured in millimeters. The different isolates have been described as susceptible/resistant based on the recommendations of National Committee for Clinical Laboratory Standards Institute (CLSI, 2013).

3. Results and Discussion

3.1 Investigation

All sales people are of Senegalese based in Brazzaville, whose age is between 30 and 45. None of the vendors have completed high school or been trained in catering. The various sales outlets are not compartmentalized, the sellers do not wear an apron or headdress and the non-respect of the principle of walking forward and the presence of a pet have been noted. The meat used is bovine in nature. Vendors get it at cold rooms in the market. They carry out with bare hands the preparation and sale of sandwiches. The preparation is similar in the different points sale visited (Figure.1), and the sale is made in parallel with that of the other merchandise of the point of sale. The sandwich is wrapped with newspaper to be served to the customer.

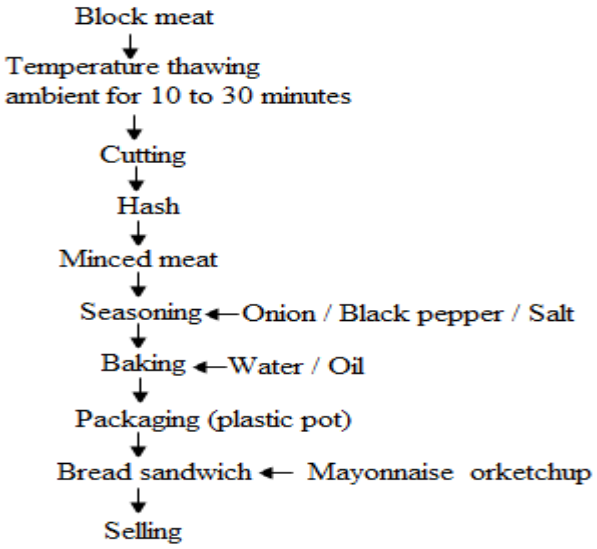

Figure 1. Diagram of preparation of minced meat sandwich

These observations highlight a lack of knowledge or negligence of basic hygiene rules by sellers. This fact has been reported for a large number of ready-to-eat foods in the informal sector in Africa, as, Kilishi, sausages, KobaRavina and dry meat (Barro et al., 2002; Mensah et al., 2002; Ranaivoarimanana, 2006; Barro et al., 2007; Badibanga, 2008; Ogunsola & Omojola, 2008; Anin et al., 2016).

3.2 Bacteriological Analysis

Results of the various analyses are presented in Table 2. The mini-feeds A, C, D and E had at least 60% non-compliant samples, compared to 20% for the B outlet. The percentages of satisfactory quality samples, acceptable and unsatisfactory, were 36%, 8% and 56% respectively. The total flora is responsible for the non-compliance of the largest number (71.43%) of samples (Figure 2). In contrast, no sulfite-reducing anaerobic bacteria, or salmonella were found in our samples.

Table 2. Bacterial load and microbiological quality of samples

Samples	FMAT	CT	CF	SSP	ASR	*Salmonella*	Results
	Norms (ufg/g)						
	3.10^5	10^3	10	10^2	30	Abs in 25g	
Site A							
B1	$7.3.10^5$	8.10^2	5	$1.3.10^1$	00	Abs	S
B2	$1.2.10^6$	10^3	18	5.10^1	00	Abs	A
B3	10^6	$3.4.10^3$	12	$4.6.10^1$	00	Abs	NS
B4	$2.2.10^6$	$1.2.10^3$	240	$2.1.10^1$	00	Abs	NS
B5	$1.2.10^6$	$1.53.10^4$	3	$4.1.10^1$	00	Abs	NS
Site B							
S1	$1.7.10^5$	$1.7.10^3$	23	$2.3.10^1$	00	Abs	S
S2	$3.5.10^5$	$2.1.10^3$	25	$1.1.10^1$	00	Abs	S
S3	$1.2.10^6$	10^3	7	$3.6.10^2$	00	Abs	NS
S4	$2.9.10^5$	$9.8.10^2$	16	7.10^1	00	Abs	S
S5	$3.6.10^5$	2.10^3	17	$2.6.10^1$	00	Abs	S
Site C							
C1	$1.29.10^6$	2.10^2	27	10^1	00	Abs	A
C2	$6.6.10^5$	$3.5.10^3$	220	$1.4.10^1$	00	Abs	NS
C3	$5.3.10^5$	$3.1.10^3$	130	$1.2.10^1$	00	Abs	NS
C4	$1.35.10^6$	3.10^2	6	$5.2.10^2$	00	Abs	NS
C5	$4.2.10^5$	$1.3.10^3$	10	$2.1.10^1$	00	Abs	S
Site D							
L1	$7.5.10^5$	$1.1.10^3$	16	$1.3.10^1$	00	Abs	S
L2	$5.8.10^6$	$3.3.10^3$	13	$1.4.10^1$	00	Abs	NS
L3	3.10^5	2.10^2	11	$2.7.10^1$	00	Abs	S

L4	$8.2.10^5$	5.10^2	35	$3.7.10^3$	00	Abs	NS
L5	$1.3.10^6$	$3.6.10^3$	11	$1.3.10^1$	00	Abs	NS
Site E							
F1	$2.9.10^6$	$3.9.10^2$	16	$4.7.10^2$	00	Abs	NS
F2	$3.3.10^6$	$2.3.10^2$	19	$4.1.10^1$	00	Abs	NS
F3	5.10^5	$4.1.10^3$	33	$2.8.10^1$	00	Abs	NS
F4	$1.2.10^6$	$1.23.10^4$	10	10^1	00	Abs	NS
F5	$1.2.10^5$	$5.4.10^2$	18	$3.4.10^1$	00	Abs	S

S: Satisfactory A: Acceptable NS: Unsatisfactory Abs: Absence

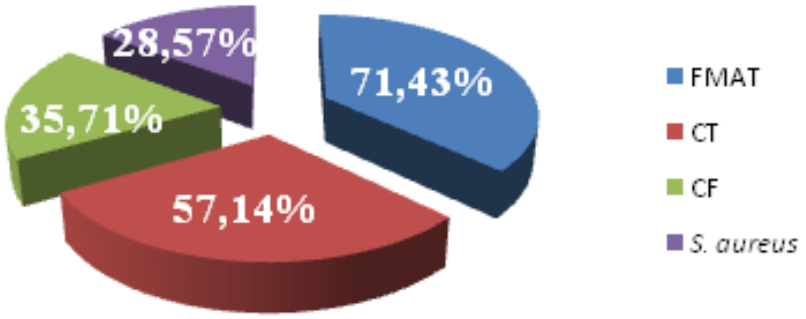

Figure 2. Proportions of non-compliance of samples by microbiological parameter

Bacteriological non-compliance of with most samples (56%) corroborates the survey results. Only outlet B has three sellers. This large workforce indicates good organization of work, which could help reduce the contamination of minced meat in this point of sale (only 20% of non-compliant samples).

The concentration of total flora beyond the limits defined for cooked minced meat can be explained by a lack of hygiene of the preparation processes or a state of putrefaction or by the preservation of the food product at a lower temperature at 65°C (Ghafir et al., 2008). The presence of total coliforms (14.29% of samples) indicates non-compliance with good hygienic practices, and that of fecal coliforms (21.43% of samples), a fecal contamination a human or animal origin. Unusually high concentration of coliforms in cooked minced meat may be explained by ineffective heat treatment or subsequent treatment contamination because coliforms are heat-sensitive.

The presence of Staphylococci in 7.14% of samples can be attributed to poor handling and hygiene practices. Since these bacteria are commensal to the skin and mucous membranes of humans, vendors can smear cooked ground meat with dirty hands, clothes or sneezing (Kotzekidou, 2013). In addition, some Staphylococci produce a heat-stable enterotoxin that may persist after heating or cooking minced meat. Finally, the absence of *Salmonella* in all cooked minced meat samples may be the consequence of the thermal effect of cooking. Gledel & Corbion (1983) reported that relatively mild heat treatments (65 °C / 15 min) are sufficient to kill *Salmonella* in contaminated food; their presence in food is therefore rare and accidental.

Dione (2000) and Ranaivoarimanana (2006), also reported a complete absence of *Salmonella* in ready-to-eat food samples sold in the informal sector, including cooked salads and Kobaravina. However, the total absence of *salmonella* in the samples should be taken with caution. In fact, *Salmonella* detection by the classical method may be falsely negative, depending on the nature of the isolation medium and the possible presence of competing microorganisms such as coliforms (Catsaras & Grebot, 1984; Ilboudo et al., 2010).

3.3 Identification of Isolated Enterobacteriaceae

Preliminary characterization of 20 isolates of cooked ground meat revealed that 85% are potentially *enterobacteriaceae.* Indeed, 17 isolates are rods, mobile, Gram positive, catalase positive and non-sporogenic. The use of the biochemical gallery confirmed their belonging to this family at the species level and, *Escherichia coli* (35.30%) was the most abundant species (Table 3).

Table 3. Results of biochemical identification

Strains	LDC	ODC	H_2S	IND	LAC	DUL	PA	UR	CIT	OX	Identification
S_3,S_5,S_6,S_8, S_{18} , S_{19}	+	+	-	+	+	-	-	-	-	-	*Escherichia coli* (35.30 %)
S_{11} , S_{15}	-	-	+	+	-	-	+	+	-	-	*Proteus vulgaris* (11.76 %)
S_{16} , S_{20}	+	-	-	+	+	+	-	+	+	-	*Klebsiella oxytoca* (11.76 %)
S_1, S_7, S_9,S_{13}	-	+	+	-	-	+	-	+	+	-	*Citrobacter* spp. (23.53 %)
S_{12} , S_{14}, S_{17}	+	+	-	-	+	-	-	+	+	-	*Enterobacter cloacae* (17.65 %)

LDC: Decarboxylation of lysine, OX: Oxidase test, IND: Indole test

UR: Hydrolysis of urea, ODC: Decarboxylation of ornithine, CIT: Use of citrate

H_2S: Production of hydrogen sulfide, LAC: Fermentation of lactose

DUL: Fermentation of dulcitol, PA: Desamination of phenylalanine

Enterobacteria include several genera and pathogenic species, their presence in cooked meats indicates a lack of hygiene (Gueye, 2007, Ghafir et al., 2008). Among the *enterobacteriaceae* isolated from cooked minced meat, *Escherichia coli* was the predominant species. According to Lachhab (2013) and Edberg et al. (2000), the presence of *Escherichia coli* in a food is an indication of the presence of enteropathogenic microorganisms, which represents it a risk to the health of consumers. Several studies have reported the presence of enterobacteria in ready-to-eat meat products (Abisa, 2004; Hamiroune et al., 2017).

3.4 Antibiotic Resistance Testing

The proportions of the sensitive and resistant strains were respectively 57.35% and 42.65%.

Intermediate responses were considered to be resistant (Table 4). The isolates showed varied resistance profiles to the different antibiotics tested and a large number of strains (48.28%) resisted the action of cefalexin (Figure 3). In contrast, nitrofurantoin expressed good activity

on enterobacterial strains, because no resistance was observed.

Table 4. Inhibition zone (mm) of antibiotics against enterobacterial strains

Species	Strains	CRO	NOR	FT	CN
Escherichia coli	S_3	S	S	S	S
	S_5	R	R	S	S
	S_6	S	S	S	S
	S_8	R	R	S	S
	S_{18}	R	S	S	R
	S_{19}	R	R	S	R
Proteus vulgaris	S_{11}	R	R	S	S
	S_{15}	R	S	S	S
Klebsiella oxytoca	S_{16}	R	R	S	R
	S_{20}	R	R	S	R
Citrobacter spp	S_1	R	R	S	R
	S_7	R	R	S	S
	S_9	R	R	S	S
	S_{13}	R	S	S	S
Enterobacter cloacae	S_{12}	R	R	S	S
	S_{14}	S	S	S	S
	S_{17}	R	S	S	S

CRO: Ceftriaxone, NOR: Norfloxacin, CN: Cefalexin, FT: Nitrofurantoin

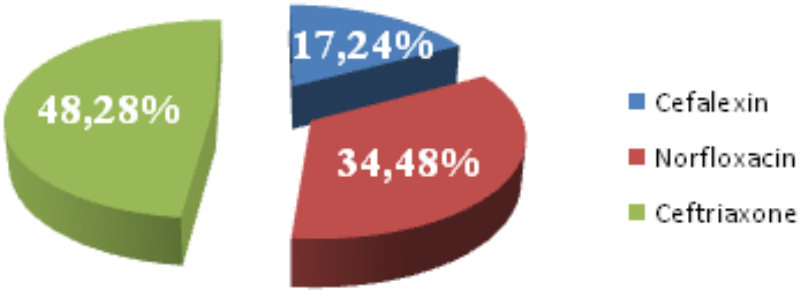

Figure 3. Proportions of resistant strains by antibiotic

The resistance to antimicrobial agents by bacterial pathogens is a major hindrance to successful therapy and some bacterial strains have been reported to be resistant to most available antimicrobial treatments (Oladipo & Adejumobi, 2010). In this study, enterobacterial strains (42.65%) were resistant to cefalexin, ceftriaxone and norfloxacin. The first two antibiotics belong to the β-lactam family. Robin et al., (2012) and Okalla et al., (2015) reported that most enterobacterial species naturally harbor and have resistance to β-lactams, as they produce β-lactamases. These enzymes hydrolyze β-lactams by inactivating the antibiotic in question (Ambler, 1991). The resistance of enterobacteria to fluoroquinolones, such as norfloxacin, is mainly due to a lack of affinity of the target and more rarely to efflux phenomena (Merensa & Servonneta, 2010). *Enterobacteriaceae* have an obvious ability to acquire and exchange genes that carry resistance factors and the intestinal flora provides an extraordinary opportunity for the flow of genetic information between bacteria (Van Immerseel et al., 2004). This frequent acquisition of antibiotic resistance mechanisms explains that *Enterobacteriaceae* are the bacteria most often implicated in human infectious pathology.

4. Conclusion

Ground meat sandwiches sold in the informal sector in Brazzaville can be a source of enteropathogens, which could expose consumers to food poisoning. The reduction of this health risk can be done through information sessions for sellers on health education, promotion of personal hygiene and food hygiene.

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