Microbiological Evaluation of Skewers and Grilled Beef and Chicken in Antananarivo (67ha and Ankatso Areas)

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ABSTRACT

The aim of this study was to assess the microbiological quality of beef skewers and grilled chicken available in a restricted area of Antananarivo : 67ha and Ankatso. Hygiene of the products, the types of consumers and the frequency of consumption are studied. Samples from these two areas were aseptically collected. The samples were analyzed within an hour of procurement. Isolation, enumeration and identification of the prevalent bacteria (*Escherichia coli, Staphylococcus aureus*, pathogenic germs like *Salmonella spp* and *Campylobacter jejuni*) were carried out following the standard procedures. Surveys of one hundred fifty vendors show that 57.74% are composed by men, 42.25% by women and 70.16% between the age of 18 and 50. The survey has shown that the product is mostly consumed by students (88.46% at Ankatso and 47.8% at 67 ha). The microbiological analysis show that the skewers are contaminated by *E.coli* (up to $3,3.10^3$CFU/g for the skewers and $5,3.10^3$CFU/g for the grilled chicken). Pathogenic germs as *Campylobacter jejumi*, *Salmonella spp* are observed. It shows a lack of hygiene and preparation : poor hygiene at any stage of the food chain, lack of preventive during processing and preparation of the food, incorrect use of the materials, contaminated raw materials, water.

Keywords-street food, skewers, grilled chicken, hygiene quality, microbiological quality

I.   Introduction

The field of street food has a big place in African urban life. In underdeveloped countries like Madagascar, selling street food is a source of supply to be consumed by almost the population who work all day away from home [20]. In Antananarivo city, 9 per 10 household members do not have lunch together [17]. Street food can satisfy the population's needs as it is quick and cheap [5]. Skewers and grilled chicken are some of the most consumed street foods in Antananarivo [18]. They provide a source of revenue for many families. The main risks linked this small business are the preparation and cooking hygiene, the sanitation of the sale environment, the infrastructure for the sale and the bad handling of the products [6]. Particularly in Madagascar, not respecting the right hygiene is one of the biggest problems for the public health [4]. So that, diseases provoked by contaminated and unsafe food is prevalent [15]. Recently, faced to the increase in numbers of the street food vendors; a study about the hygienic quality of street food like 'Misao', 'Oysters' and 'Koba ravina' had been done [2,19, 21]. Those studies proved the existence of food-borne outbreak germ and infectious disease from food. So, this study is to evaluate the hygienic quality of grilled chicken and beef sold around 67ha and Ankatso in Antananarivo city. The general objective of this work is to contribute to the protection of the consumers of street food. In a specific way, it is necessary to evaluate the microbiological quality of these foods, to know the hygiene of the vendors during the preparation and sale of the products.

II.   Materials and methods

2.1. Survey

The surveys were done depending on the availability of the vendors. The study took place in six (6) quarters: 67ha Center, 67ha North-West, 67ha North-East,Ankatso 119 and Ankatso-Tsiadana. We have chosen those places according to the number ofthe vendors and the frequency of the consumption. In addition, 67ha is a popular place near to the new bus station at Andohatapenaka, the bus stop to National Road 4 and studentswho circulate there. Ankatso has been selected for this study to check the foods served tothe students and workers at the university.

2.2. Sampling

We have also applied random stratified sampling during the work. Vendors in the twostudy areas were divided into two sections related to the number of quarters. We selected five vendors in each zone to get five samplings from each of them (n=5). We have chosen thismethod to represent all quarters in the areas. Sixty (60) samplingswere chosen for the collection of samples: 30 beef skewers and30 grilled chicken. All the samples were aseptically collected in sterile containers, stored at 4°C and analyzed within an hour of procurement.

2.3.Microbiological analysis

- Enumeration of Total Aerobic Bacteria
  Plate Count Agar (PCA) (Oxoid Ltd, United Kingdom) was used for Total Aerobic Bacteria and wasdone in conformity with the recommendation of the norm ISO 4833 (7, 21).
- Detection of *Salmonella spp.*
  Salmonella sppwas detected with the recommendation of the norm ISO 6579. Twenty-five grams (25g) of each sample was mixed with 225 ml of buffered peptone water and incubated at 37oC for 16 h.One ml of this culture was pipetted into 10 ml of Rappaport-VasilliadisSoya broth (RVS). These wereincubated at 41oC for 24 h. The culture was streaked intoHektoen Agar. The agar plate wereincubated at 37oC for 24 h. The plate were examined for typical green blue colonies of *Salmonella* (8,16, 19, 21).
- Detection of *Escherichia coli* βglucuronidase +
  1 ml of the dilution of each food sample was plated onto Eosin Methylen Blue Agar Medium andincubated at 44°C for 24h to 48 h. Black green metallic colonies were subjected to appropriatebiochemical tests according to the norm ISO16649(11,14).
- Detection of *Campylobacter jejuni*
  25 g of the food sample was mixed with 100 ml Preston broth (Oxoid) and homogenized for 2 min.The enrichment broth was incubated at 42°C for 24 to 48 h. The broth culture was streaked ontoSkirrow's agar plates (Oxoid), which were then incubated at 42°C. Colonies were Gram stained andtested for oxidase reaction. Suspect colonies were subjected to appropriate biochemical tests, done inconformity with the recommendation of the norm ISO 10272: 2006 (10, 21).

2.4.Statistical analysis

XLSTAT was used for the statistical analysis of results. The Main Component Analysis (MCA) permit to know the most contaminated zone and the most incriminated microorganism.

III.   Results

3.1. Surveys

The surveys about the respect of hygiene in the sampling sites permitted to show the state of vendors and dwellers, the way of conservation of the products and the environment in theselling points. In total, 150 vendors around 67ha and Ankatso were surveyed.

- **Vendors :** most of them are between the age of 18 and 50 (70.16%, 57.74% are men and 42.25% are women, only 20% have attended high school education, 65 % have completed the primary and secondary education, and 15% are illiterate. Figure 1 shows knowldges of the vendors about hygiene, during the preparation and sale of the products.
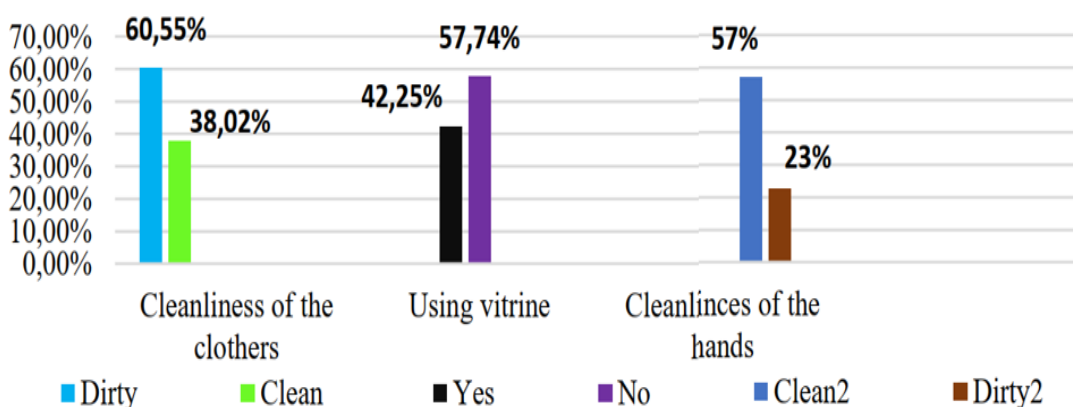

Figure 1 : Hygiene of vendors

- **Consumers**: In total, 300 consumers were interviewed around 67ha and Ankatsoincluding 68.64% men and 31.35% women. Their age is between 16 and 54. The majority of the consumers of the products is student: 88.46% at Ankatso and 47.88% at67ha.Skewers are mainly consumed with beverages (53.49%). Results show that 10.16% of people consume the product three times a week, 17.79% consume it twice a week, 26.27% once a weekand the rest consume it occasionally. Skewers and grilled chicken are especially consumedduring parties (44.27%) and on week-ends (33.58%).According to the survey results, 10.45% of the consumers told that they got digestive disorderfrom the product and 20.55% could not confirm the cause of their discomfort after eating theskewers.

- In total, 300 consumers were interviewed around 67ha and Ankatso including 68.64% men and 31.35% women. Their age is between 16 and 54. The majority of the consumers of the products is student: 88.46% at Ankatso and 47.88% at 67ha. Skewers are mainly consumed with beverages (53.49%). The consumers surveyed (10.16%) consume the product three times a week, 17.79% consume it twice a week, 26.27% once a week and the rest consume it occasionally. Skewers and grilled chicken are especially consumed during parties (44.27%) and on week-ends (33.58%). According to the survey results, 10.45% of the consumers told that they got digestive disorder from the product and 20.55% could not confirm the cause of their discomfort after eating the skewers. This next figure shows us the identified symptoms of consumers.
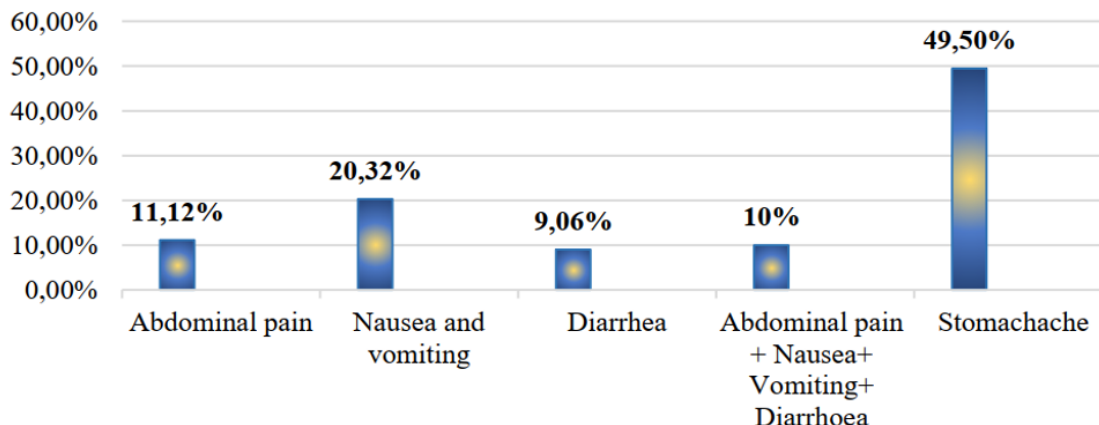
Figure 2 : Identified sypmptoms

- Five types of symptoms were identified during the interview: 49.50% are stomachache, 20.32% are nausea and vomiting, 11.12% are abdominal pain, 9.06% are diarrhoea and 10%got the three last symptoms.

3.2. Results of the microbiological analysis

The average value of the germ analysis results in the skewers and grilled chicken taken in the six quarters is conclude in this following table.

Table 1 : The average value of the microbiological results in the skewers

	Beef skewers samples (n=5)						Criterias (CE n°02007 3/ 2005)	Quality
	South 67ha	67 ha Center	67ha North-West	67ha North-East	Ankatso-119	Ankatso-Tsiadana		
FMAT (CFU/g)	$1,1.10^6$a	$8,6.10^5$a	$9,3.10^5$a	$1,04.10^6$a	$1,3.10^6$a	2.10^6a	m= 3.10^5 M= 3.10^6	Ac
E. coli (CFU/g)	$3,05.10^3$a'	6.10^2a'	$2,4.10^3$a'	$2,2.10^3$a'	$1,7.10^3$a'	$3,3.10^3$a'	m= 1.10^2 M= 1.10^3	U
S. aureus (CFU/g)	$1,5.10^4$a''	$4,06.10^3$a''	$1,4.10^3$a''	$3,4.10^3$a''	$3,2.10^3$a''	$1,08.10^3$a''	m= 1.10^2 M= 1.10^3	U
Salmonella spp (CFU/g)	3/5 (60%)	1/5 (20%)	2/5 (40%)	2/5 (40%)	3/5 (60%)	3/5 (60%)	Abs/25 g	U

TAMF : Total Aerobic Mesophilic Flora ; E.coli : Echerichia coli β-D-glucuronidase positive ; S. aureus : Staphylococcus aureus ; AC : acceptable ;U : unsatisfying ; CFU/g : Colony-Forming Units per gram of sample ; n=numbers of samples ; Abs 25 g : absence in 25 g of sample.

The numbers with the same letter in one column are not significantly different (p 0.05). The table 2 indicates that the microbiological quality of the beef skewers in the six quarters is acceptable in TAMF, unsatisfying in *E. coli* and *S. aureus*. We have also noticed the presence of *Salmonella spp* in the skewers which shows an unsatisfying result of the microbiological quality.

Table 2 : The average value of the microbiological results in the grilled chicken
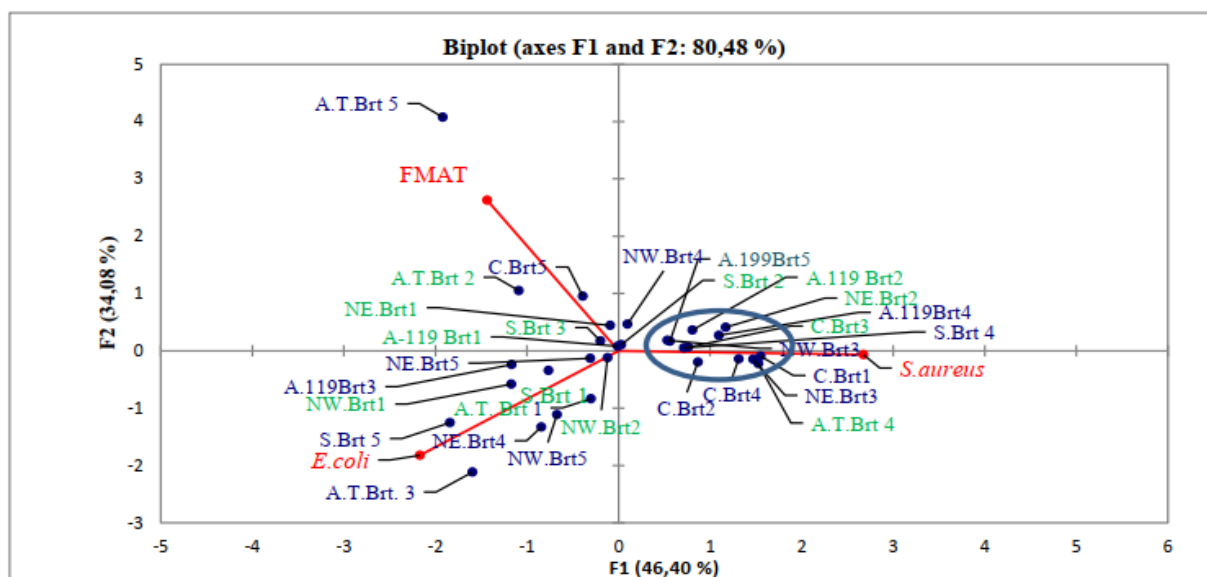
	South 67ha	67 ha Center	67ha North-West	67ha North-East	Ankatso-119	Ankatso-Tsiadana	Criterias (CE n°020073/ 2005)	Quality
	Grilled chicken samples (n=5)							
TAMF (CFU/g)	$7,1.10^5a$	$1,04.10^6a$	$9,6.10^5a$	$1,2.10^6a$	7.10^5a	$2,4.10^5a$	$m= 3.10^5$ $M= 3.10^6$	Ac
E. coli (CFU/g)	$4,03.10^3a'$	$5,3.10^3a'$	$1,6.10^3a'$	$4.10^3a'$	$1,3.10^3a'$	$2.10^3a'$	$m= 1.10^2$ $M= 1.10^3$	U
S. aureus (CFU/g)	$4,03.10^3a''$	$3,6.10^3 a''$	$2.10^3 a''$	$3,03.10^3 a''$	$3,3.10^3 a''$	$2,3.10^3 a''$	$m= 1.10^2$ $M= 1.10^3$	U
Salmonella spp (CFU/g)	0/5 (0%)	1/5 (20%)	2/5 (40%)	2/5 (40%)	3/5 (60%)	4/5 (60%)	Abs/25 g	U
C. jejuni (CFU/g)	0/5 (0%)	1/5 (20%)	2/5 (40%)	2/5 (40%)	1/5 (20%)	4/5 (80%)	Abs/25 g	U

TAMF : Total Aerobic Mesophilic Flora ; E.coli : Echerichia coli β-D-glucuronidase positive ; S. aureus : Staphylococcus aureus ; AC : acceptable ;U : unsatisfying ; CFU/g : Colony-Forming Units per gram of sample ; n=numbers of samples ; Abs 25 g : absence in 25 g of sample.

The numbers with the same letter in one column are not significantly different (p 0.05). The table 3 indicates that the microbiological quality of the grilled chicken in the six quarters is acceptable in term of TAMF, unsatisfying in term of *E. coli* and *S. aureus*. Those results are sign of the bad quality of the products because of the lack of hygiene of the used raw materials and the personnel. Contamination from the pathogen germs: *Salmonella spp* and *Campylobacter jejuni* was noticed in five quarters among the six studied area. So, the microbiological quality of the grilled chicken is insufficient.

3.3. Results of the statistical analysis
Principal Component Analysis (PCA)

A principal component analysis is done to study the correlation between the germs, to compress information and represent the samples in the form of points in a reduced space. The PCA of the germs in the beef skewers is illustrated in figure 3.
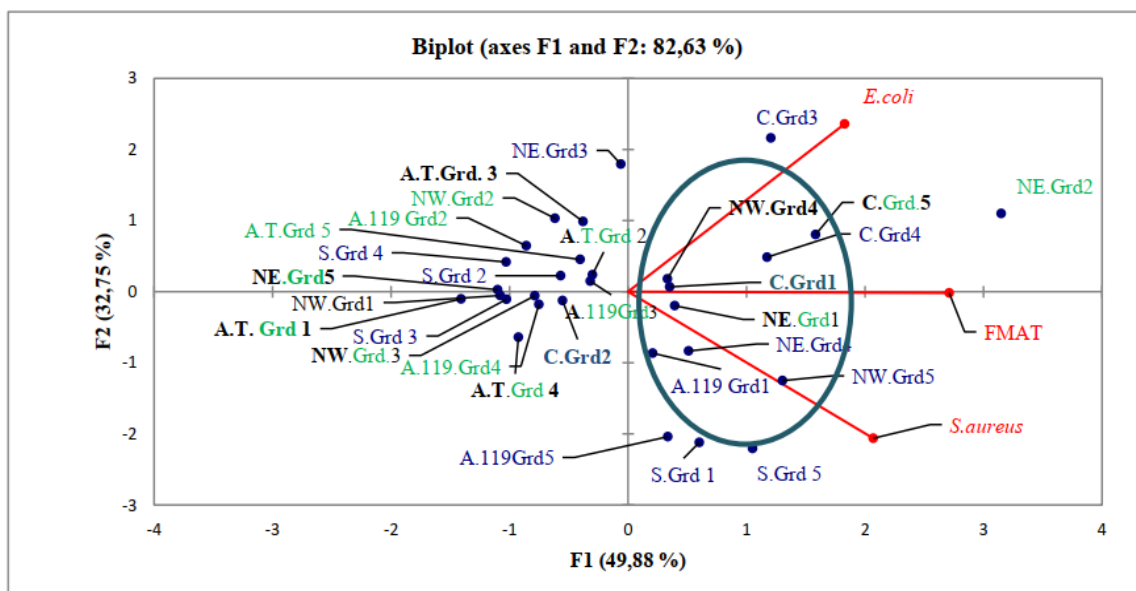

S (67ha south) ; C (67ha center) ; NW (67ha north-west) ; NE (67ha north-east) ; A(Ankatso 119) ; A.T(Ankatso-Tsiadana) ; Brt (skewers) ; the number of the sample is put in index 1, 2, 3, 4, 5 (for exemple : S.Brt.1 ; a sample of beef skewer taken from the quarter at 67ha Sud).

Figure 3 : Germs in the beef skewers

The first factorial axis (Dm1) constitutes 46.40% of the total variance and the second factorial axis (Dm2) covers 34.06%. The variability sum represents 80.48% of the total variability of the dataset. It means the factorial axis has a right variability and respect the 75% standard variability. The first factorial axis is negatively correlated to the TAMF and *E. coli* variables while its correlation to *S. aureus* is positive. The second factorial axis implies with the TAMF. The different samples were divided into two factorial axes to evaluate the germ concentration in the skewers. The sample "A.T.Brt5" is positioned at the left of the figure. It shows a strong contamination by all the studied germs. The lot circled in blue on the factorial axis (Dm1) is the samples more contaminated by *S. aureus*: four samples from 67ha Center, three samples from Ankatso 119 and two samples from 67ha North-west. The lot circled in red are the samples more contaminated by *E. coli* but they are less contaminated by *S. aureus*: three samples from 67ha North-west, two samples from Ankatso Tsiadana, South 67ha and 67ha North-west. Each quarter represents *salmonella spp* contamination but the most contaminated were the samples from Ankatso Tsiadana and South 67ha (the samples contaminated by *Salmonella spp* are green colored).The PCA of the germs in the grilled chicken is shown in the figure 4.

Figure 4 : Germs in the germ in the grilled chicken

S (67ha south) ; C (67ha center) ; NW (67ha north-west) ; NE (67ha north-east) ; A(Ankatso 119) ; A.T(Ankatso-Tsiadana) ; Grd (Grilled chicken) ; the number of the sample is put in index 1, 2, 3, 4, 5 (for exemple : S.Grd.₁ ; a sample of grilled chicken taken from the quarter at 67ha Sud).

In the figure 4, the first factorial axis (Dm1) constitutes 49.87% of the total variance. The second axis (Dm2) explains the 32.75%. These two components can prove that 82.63% of the total variable were from the dataset. All the variables are positively correlated with the first main factor. The blue circled samples situated at the right part of the factorial axis (Dm1) are mainly contaminated by *S. aureus*, *E. coli* and TAMF. They represent four samples from 67ha Center and three samples from 67ha North-East. *Salmonella spp* is detected and *Campylobacter jejuni* (black colored) are suspected in the green colored samples. There was no sample contaminated by those two pathogen germs in the zone of South 67ha.

IV. Discussion

According to the collected data, the hygiene notion and necessary skills for the right hygiene are already known by the merchants, but it is not respected for some of them. About the disease symptoms, we could identify the presence of these following germs: *Salmonella spp, Campylobacter jejuni, E.coli and S. aureus.* The general average concentration of TAMF in the two types of samples is 1,2.106 UFC/g for the beef skewers and 8,1.105 UFC/g for the grilled chicken. So, the results are basically acceptable for a three-class plan. All the analyzed samples are not consistent with the microbiological criterias of *E. coli* and *S. aureus.* The medium charge of *E. coli* is 2,2.103 UFC/g for the beef skewers and 2,6. 103 UFC/g for the grilled chicken. A high concentration of *E. coli* is provided from fecal contamination during killing the animals and transporting the meat. As defined in the accomplished study, the contamination of fresh meat sold in the open market by the germ of *E. coli* is up to 1,13.103UFC/g [18], which means that the meat are already contaminated. The average concentration of *S. aureus* is 2,6.103 UFC/g for the beef skewers and 3,03.103 UFC/g for the grilled chicken. It is due to the inconsistency of the selling point: the meat that are ready to grill are exposed on a table without protection. *S. aureus* is a pathogen germ but also a hygiene indicator in case of handling preparation of the products [7,9]. All foods may be contaminated by *Salmonella spp.* They are some of the main bacterial infectious agent of foodborne infection [16]. The obtained result is alarming as 53.33%

of the beef skewers and 36.66% of the grilled chicken are contaminated by *Salmonella spp*. Inaddition, 43.33% of the grilled chicken contains *Campylobacter jejuni*.So that, the two products are considered unsatisfying and all the results are mostly related tothe lack of hygiene during the preparation of the meat [8].

V. Conclusion

The sector of street foods has a big place in city life. It brings a lot of interest for themerchants and for the customers. According to microbiological analysis result, it has beenproved that the quality of skewers and grilled chicken around 67ha and Ankatso is insufficient.These foods have less satisfying quality of *E. coli* an *S.aureus*. The skewers are contaminatedby *Salmonella spp*. Two pathogen germs were observed in the grilled chicken (*Campylobacterjejuni* and *Salmonella spp*). So that, it is necessary to share skills to the merchants about foodhygiene by educating or training them. Experienced also should be shared to promote a rightstrategy for them to get safe food for the population.

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