Microbiological quality of selected street foods from Antananarivo on 2016-2017: public health implications

Tsirinirindravo H.L.[1], Randrianierenana L.A.[1], Andrianarisoa B.[1], Raherimandimby M.[1], Rakotoarisoa M.T.[1], Andriamady H.[1], Randriamiarisoandraibe H.[1],Andriampenotanjona B. F.[1], De Percin G.[2], Delandes X.[2], Pierluigi B.[3], Larroque M.[4], Margout D[4].

1. Département de Biochimie fondamentale et appliquée, Faculté des Sciences d'Antananarivo, Madagascar
2. Ecole spéciale ESTPI, Paris
3. International University Network on Cultural and BiologicalDiversity (IUNCBD), Italie
4. Faculté de Pharmacie de Montpellier, France

ABSTRACT

People in a big city as Antananarivo, capital of Madagascar, have leads to take street foods for their daily nutritional needs. This food habits may be a risk for consumers due to contaminations from street environment and bad practices related to hygiene. This study aimed to examine the quality and safety of street vended foods in Antananarivo, on January 2016 to December 2017.Six hundred and sixty two samples including 126samples of melting salads, 70 beef skewers, 54 chicken skewers, and typical Malagasy foods as : mofoanana (67 samples), mofogasy (64 samples), ramanonaka (64), makasaoka (66), mofoakondro (62) and kobandravina(89);were randomly collected from the streetvendors in Antananarivo marketsto evaluate their bacteriological quality.International Methods (ISO) was adopted for to find the load of Total Aerobic Bacteria andEnterobateriaceae,*Escherichia coli* and to search pathogen bacteria as *Salmonella, Campylobacter jejuni, Escherichia coli* O157H7 and *Bacillus cereus* in these foods.The results revealed that the mean values ofthe Total Aerobic Bacteria count was $0.1x10^6$- $4.8x10^6$cfu/g. *Enterobacteriaceae*count range from $0.4x10^2$ to $1.9x10^2$cfu/g. *Escherichia coli* count range from $0.04x10^2$cfu/g. to 0.19 $x10^2$cfu/g.*Salmonella*was only present in melting salads, beef skewers and chicken skewers samples. *Bacillus cereus* count range from $0,1x10^2$ to $1,5x10^2$cfu/g. *Campylobacter jejuni*was only present in samples of ramanonaka and kobandravina. Two strains of presumptive *Eschercichia coli* O157 H7 (βglucuronidase -) were isolated. PCR method was used to confirm the identity of these two isolates. A high contamination above 10^6 cfu/g food and the presence of potential pathogens bacteria could be hazardous. Systematic inspections and training of food vendors on food hygiene and application of hazard analysis critical control point (HACCP) has been recognised as measures to guarantee improvement of the quality of street foods.

Keywords: *streetfoods, Escherichia coli,E. coli O157H7, food borne diseases, Antananarivo, Madagascar, Campylobacter, Bacillus cereus.*

I. INTRODUCTION

Street-vended foods are ready-to-eat foods prepared and sold by vendors on streets or public places for fast consumption (14).

The street food is a growing sector in many developing countries. They provide a source of inexpensive and nutritional meals, although providing a source of income for the vendors (29).

Anyway, street vended food products may represent a risk due to inadequate personnel hygiene of vendors, the bad condition at which it produced, in as using raw materials of bad quality. Such contamination may render the product of inferior quality or unfit for human consumption (31*).*
It has been shown that Street-vended foods have been implicated in outbreaks of foodborne illnesses all around the world. In Madagascar, there was three food bornediseases due to *Salmonella typhi*reported on 2015-2016 and*Escherichia coli* was identified as responsible of so many infections and toxi-infections on 2017 (31, 33).

Escherichia coli, Salmonella and *Campylobacter jejuni*have recognized as a serious bacteria pathogen and has been associated with several out breaks of disease. In Madagascar, there is no available data about these bacteria and street foods. Therefore, this study was led to evaluate the microbial quality of street vended foods in Antananarivo, capital city of Madagascar on January 2016 to December 2017.

II. MATERIAL AND METHODS

Collection of samples

Six hundred and sixty two samples including 126 samples of melting salads, 70 beef skewers, 54 chicken skewers, and typical Malagasy foods as :Mofoanana (67 samples), Mofogasy (64 samples), Ramanonaka (64), Makasaoka (66), Mofoakondro (62) and Kobandravina (89);were randomly collected from the streetvendors in Antananarivo markets. Samples were sent to the laboratory within two hours after collection in a cold-box containing ice-blocks. Characteristics and nature of each food are presented in Table 1.

Table I: Description of foods analysed

Food	Nature	Description
Melting salads	Solid Mixed : cooked and fresh	Salted food, Melting salads composed by spaghetty, vegetables, Minced meat
Beef skewers	Solid Smoked	Beefsmokedpiece
Mofoanana	Solid Cooked	Typically Malagasy foods composed by vegetable, Brede fritter
Makasoka	Solid Cooked	Typically Malagasy foods : a kind of French toast
Mofogasy	Solid Cooked	Typically Malagasy foods:Sweet fried food made of rice flour.
Ramanonaka	Solid Cooked	Typically Malagasy foods : salted fried food made of rice flour
Mofoakondro	Solid Cooked	Sweet banana fritter
Chicken skewers	Solid Smoked	Chickensmokedpiece
Kobandravina	Solid Cooked	Typically Malagasy foods : a kind of pudding made of peanuts, flour, sugar,grinned and cooked inbanana leaves

III. Sample preparations and analysis

Serial dilution

Twenty-five grams (25 g) of each sample was mixed carefully with 225 ml of buffered peptone water. This mixture was homogenized and shacked to obtain a uniform mixture. One ml of the homogenized

food sample was aseptically transferred into a test tube containing 9 ml sterile distilled water. Five dilutions of the homogenates were prepared in conformity with the recommendation of the norm ISO 6887 (6).

Enumeration of Total Aerobic Bacteria

Plate Count Agar (PCA) (Oxoid Ltd, United Kingdom) was used for Total Aerobic Bacteria and was done in conformity with the recommendation of the norm ISO 4833 (7).

Enumeration of *Bacillus cereus*

The recommendation of the norm ISO 7932 was used. 1 ml of the dilution of each food sample was plated onto polymyxin-pyruvate-egg yolk mannitol-bromothymol blue agar plates (Oxoid), which were air dried and incubated at 37°C for 24 to 48 h. Blue colonies with blue zones were subjected to appropriate biochemical tests (9,18).

Detection of *Salmonella* spp.

*Salmonella spp*was detected with the recommendation of the norm ISO 6579. Twenty-five grams (25 g) of each sample was mixed with 225 ml of buffered peptone water and incubated at 37^{o}C for 16 h. One ml of this culture was pipetted into 10 ml of Rappaport-VasilliadisSoya broth (RVS). These were incubated at 41^{o}C for 24 h. The culture was streaked intoHektoen Agar. The agar plate were incubated at 37^{o}C for 24 h. The plate were examined for typical green blue colonies of *Salmonella* (8, 16, 19).

Detection of *Escherichia coli* *β*glucuronidase +

1 ml of the dilution of each food sample was plated onto Eosin Methylen Blue Agar Medium and incubated at 44°C for 24h to 48 h. Black green metallic colonies were subjected to appropriate biochemical tests according to the norm ISO16649(11,14).

Detection of *Escherichia coli* O157:H7

This strain was determined using sorbitol MacConkey agar (Oxoid) plates. *Escherichia coli O 157 H7* doesn't use sorbitol and gives characteristics colonies on this medium. Then, strains suspects belonging to *E. coli* O157H7 must be identified by PCR, using Kit BAX (Qualicon, Inc. - USA) for screening *Escherichia coli* O157H7 with a detection rate around 96,5 % (12, , 14, 16).

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 onto Skirrow's agar plates (Oxoid), which were then incubated at 42°C. Colonies were Gram stained and tested for oxidase reaction. Suspect colonies were subjected to appropriate biochemical tests, done in conformity with the recommendation of the norm ISO 10272: 2006 (10).

IV. RESULTS

As shown in Table 2, melting salads, beef skewers, chicken skewers and kobandravina were found to be contaminated. A high level of Total Aerobic Bacteria TAB (>10^6ufc/g),*Enterobacteriaceae*(>10^2/g) and *Escherichia coli* β glucuronidase + is noted.

The values ofthe Total Aerobic Bacteria count was 0.1x10^6- 4.8x10^6cfu/g. *Enterobacteriaceae*count range from 0.4x10^2 to 1.9x10^2cfu/g and *Escherichia coli* count range from 0.04x10^2cfu/g. to 0.19 x10^2cfu/g.

Pathogen bacteria as *Salmonella* was only present in melting salads, beef skewers, chicken skewerssamples. *Bacillus cereus* count range from 0,1x10^2 to 1,5x10^2cfu/g. *Campylobacter jejuni*was only present in samples of beef and chicken skewers. Two strains of*Eschercichia coli* O157 H7 (βglucuronidase -) were isolated and identified by PCR reaction from beef skewers.

Table 2: Microbiological assessment of street foods samples collected in Antananarivo market on 2015-2016.

Number	Samples	TAB. 10^6/g	Ent. 10^2/g	E.C.BG+ 10^2/g	E.C.BG- /g	SLM /g	CAMP /g	BC 10^2/g
126	Melting salads	4,854	1,927	1,259	A	12,49	A	1,445
70	Beef skewers	3,327	1,72	1,19	2	20,93	0,188	A
67	Mofoanana	0,424	0,785	0,04	A	A	A	0,572
66	Makasoka	0,182	0,489	0,09	A	A	A	0,574
64	Mofogasy	0,517	0,933	0,198	A	A	A	0,299
64	Ramanonaka	0,459	0,474	0,147	A	A	A	0,172
62	Mofoakondro	0,311	0,555	0,067	A	A	A	0,374
54	Chicken skewers	3,998	1,961	1,338	A	2,06	0,942	A
89	Kobandravina	1,585	1,199	1,011	A	A	A	1,553

TAB : Total Aerobic Bacteria
Ent :Enterobacteriaceae
E.C.BG + : *Eschercichia coli βglucuronidase* +
E.C.BG - : *Eschercichia coli βglucuronidase* -
SLM :*Salmonella spp*
CAMP :*Campylobacter jejuni*
BC :*Bacillus cereus*
A: Absent

V. DISCUSSION

The result of these different analysis carried out on street foods samplesrevealed that all samples collected were contaminated by microorganisms.This is due to the inadequate personnel hygiene of vendors, the bad condition at which it produced, and using raw materials of poor quality or the fact that they were exposed in an open air because there are several microorganisms (beneficial or pathogen) that we can find in environment (25, 34, 35).

If they are beneficial microorganisms, it is even profitable for the food because it allows to protect them, improve their tastes, their qualities (21). However, for harmful microorganisms, this could have impacts on the food (preservation) and on the consumers.

A high level Total Aerobic Bacteria TAB (>10^6/g) was reported on melting salads, beef skewers, chicken skewers and kobandravina. Its shows a general contamination. The TAB amount allowed to appreciate the general hygiene of the product (from raw material to storage and selling conditions) (31, 35).

Bacillus cereus is only present in highly concentration in melting salads and kobandravina. Their microbial load are superior to the bacteriological criteria. It could be due to the characteristic of this bacteria to metabolize starch while these foods are made of starch.

Enterobacteriaceae is a bacillus Gram negative family. A huge species as *Escherichia coli, Salmonella, Shigella, Klebsiella …*belong to this family.Due to table 2, melting salads, beef skewers, chicken skewers and kobandravinahave a high level of *Enterobacteriaceae*. It shows a faecal contamination of the product from the vendor, form the raw material or during the preparation.However, there are extremely pathogen species in this familyandassociated with many

cases of food borne diseases (31). However, they could easily be resistant on many antibiotics, which could train a treatment failure (30, 31,32).

Salmonella species belongs to the family of *Enterobacteriaceae*. It is only present in melting salads, beef and chicken skewers. It confirms study led on 2016, which showed especially the implication of melting salads and skewers for several cases of Typhoid fever in Madagascar (29, 31).

Escherichia coli is a bacteria that normally lives in the intestines of people and animals. There are many different types of *E. coli*. Most E. coli are found naturally in intestines and play an important role in helping our bodies digest food. However, a few types of *E. coli* can cause diarrhoea and other illnesses when swallowed.

A previous study led in the Urban Commune of Antananarivo, Health Ministry and WHO shows that this species is the first responsible for foodborne disease in Antananarivo on 2016. Indeed, 14 TIAC cases had been noticed in the capital city (29).In fact, melting salads, beef skewers, chicken skewers and kobandravinaare the most contaminated food by this germ. Their consumption could train illness as diarrhoea, dysentery. In 2015, *Tsirinirindravo and al* found that melting salads was the first food associated with foodborne illness in Antananarivo. However, it is very appreciated by consumers (29, 31).

Two species extremely pathogens were found in these street foods:*Campylobacter jejuni*and *Escherichia coli* O157 H7.

E. coli O157:H7 is a toxin producing bacteria that causes intestinal disease in people which lasts about one week. Diarrhoea with blood is typical. Haemolytic uremic syndrome (HUS) is a severe complication of *E. coli* O 157 H7 infection (24).

This germ is only present in beef skewers.Most of the times, it is present in meat or hamburger. *Escherichia coli* O157H7 is noted to be responsible of more than 20000 foodborne diseases per year in the United States (24, 33).

*Campylobacter jejuni*was only present on the two skewers. Infection with *C. jejuni* usually results in enteritis, which is characterised by abdominal pain, diarrhoea, fever, and malaise. Diarrhoea itself can vary in severity from loose to bloody stools (20, 23, 34).

VI. Conclusion

The study aims to determine the microbial quality of pre-cut ready-to-eat vegetable salads sold by food vendors in the Antananarivo markets on 2015-2016.

The most contaminated food are melting salads, beef skewers, chicken skewers and kobandravina. The typical Malagasy food as Mofogasy, Ramanonaka, Menakely are the healthiest, referring to their sanitary hygienic quality.

Melting salad, chicken skewers, beef skewers and kobandravina constitute a health risk to consumers, in terms of microbial quality. The contamination could come from unhygienic food preparation, process, environmental conditions, raw materials and improper food handling. Therefor a research should be carried out to determine the antimicrobial susceptibility of the bacteria identified, Street vendors must be trained about hygiene, Good Practice hygiene GPH. The HACCP system have to be build up for street vendors.

REFERENCES

[1.] ACHYUT A., KAREN K. Effect of storage time and temperature on the viability of *E. coli O157:H7, Salmonella spp., Listeria innocua, Staphylococcus aureus, and Clostridium*sporogenes vegetative cells and spores in vacuum-packed canned pasteurized milk cheese. Journal of Microbiology. July 2018, Volume 56, Issue 7, pp 450–459

[2.] BIBEK R., Fundamental Food Microbiology 2nd ed. The CRC Press Ltd Washington, DC 2001, 56-90).

[3.] CHUKWUEMEKA, NNENNE I.I. and CHRISTIAN U.I., Bacteriological quality of foods and water sold by vendors and in restaurants in Nsuka, Enugu State, Nigeria: A comparative study of three Microbiological methods, Journal of Heath Population and Nutrition, 29(6), 2011, 560-566.

[4.] EUN-JIN L., CHOI, J., GROISMAN, E.A. 2014. Control of a *Salmonella* virulence operon by proline-charged tRNApro. Proc. Natl. Acad. Sci. USA 111, 3140–3145.

[5.] ISLAM M., DOYLE M.P., PHATAK S. C., MILLNER P., JIANG X. "Survival of *Escherichia coli* 0157:H7 in Soil and on Carrots and Onions Grown in Fields Treated with Contaminated Manure Composts or Irrigation Water." Food Microbiol. 2005. 22: 63-70.

[6.] INTERNATIONAL STANDARD ORGANISATION. Microbiologie des aliments. Préparation des échantillons, de la suspension mère et des dilutions décimales en vue de l'examen microbiologique. ISO 6887-1:1999.

[7.] INTERNATIONAL STANDARD ORGANISATION. Microbiologie des aliments. Méthode horizontale pour le dénombrement des micro-organismes. Technique de comptage des colonies à 30 degrés. ISO 4833:2003.

[8.] INTERNATIONAL STANDARD ORGANISATION. Microbiologie des aliments. Méthode horizontale pour la recherche des *Salmonella spp*. ISO 6579:2002.

[9.] INTERNATIONAL STANDARD ORGANISATION. Microbiologie des aliments. Méthode horizontale pour le dénombrement de *Bacillus cereus* présomptifs. Technique par comptage des colonies à 30 degrés C. ISO 7932:2004.

[10.] INTERNATIONAL STANDARD ORGANISATION. Microbiologie des aliments. Méthode horizontale pour la recherche et le dénombrement de *Campylobacterspp*. ISO 10272:2006

[11.] INTERNATIONAL STANDARD ORGANISATION. Microbiologie des aliments. Méthode horizontale pour le dénombrement des *Escherichia coli* bêta-glucuronidase positive. ISO 16649 :2001.

[12.] INTERNATIONAL STANDARD ORGANISATION. Microbiologie des aliments. Méthode horizontale pour la recherche des *Escherichia coli* O157. ISO 16654:2001.

[13.] FOOD AND AGRICULTURAL ORGANIZATION. 1997 .Agriculture food and Nutrition for Africa. A resource book for teachers of Agriculture, Rome. 12 (3):25-29.

[14.] FOOD AND AGRICULTURE ORGANIZATION FAO.1992. *Escherichia coli* and other coliforms. Manual of food quality control. Rev.1- Microbiological Analysis, Food and Agriculture Organization of the United Nation, Rome, Italy, Chap. 3PP, 13-26.

[15.] FOOD AND DRUG ADMINISTRATION "FDA" 2001. *Staphylococcus aureus*. Bacteriological analytical manual .8th Ed. Chapter12. Gaithersburg, p.562.

[16.] FRANZ E., BRUGGEN A. H. 2008. Ecology of *E. coli* 0157:H7 and *Salmonella enterica* in the Primary Vegetable Production Chain." Crit. Rev. Microbiol. 34 (3-4): 143-161.

[17.] GILBERT R., DE LOUVOIS J., DONOVAN T., LITTLE C., NYE K., RIBEIRO C. D., RICHARDS J., ROBERTS D., BOLTON F. B. 2000. Guidelines for the microbiological quality of ready-to-eat foods sampled at point of sale. Commun. Dis. Public Health 3:163–167.

[18.] GRANUM P. E., ANDERSSON A., GAYTHER V., GIFFEL TE M., LARSEN H., LUND T. O., O'SULLIVAN K. 1996. Evidence for a further enterotoxin complex produced by *Bacillus cereus*. FEMS Microbiol. Lett. 141:145–149

[19.] JONGHYUN B., EUNNA C., EUN-JIN L. A rule governing the FtsH-mediated proteolysis of the MgtC virulence protein from *Salmonella entericaserovarTyphimurium*. Journal of Microbiology, August 2018, Volume 56, Issue 8, pp 565–570

[20.] LARPENT J-P., LARPENT-GOURGAUD M. Mémento technique de microbiologie, 3éd. Londres, New York, Paris. Lavoisier, 1997 : 1039 p.

[21.] MANANJARA P., TSIRINIRINDRAVO H.L., RAHERIMANDIMBY M., RANDRIANIERENANA A. Etude des levures endogènes d'*Evodiabilahe*, rutacée endémique de Madagascar. Int. J. Biol. Chem. Sci. 10 (4): 1694- 1701. August 2016.

[22.] NICHOLS S.L., LITTLE C.L., MITHANI V., LOUVOIS J., The Microbiological quality of cooked rice from restaurants and take-away premisis in the United Kingdom, Journal of Food protection,62, 1999, 877-882.

[23.] PRESCOTT L.M., KLEIN D.A. (2008). Pathogenic Organisms. Microbiology, 7th ed McGrew hill, New York. pp 340, ISN 978-0-07-110231-5.

[24.] RANGEL J. M., SPARLING P. H., CROWE C., GRIFFIN P. M., SWERDLOW, D. L. 2005. Epidemiology of *Escherichia coli* O157: H7 Outbreaks in the United States from 1982 to 2002. Emerg. Infect. Dis.11 (4): 603-609.

[25.] RASOLONIAINA L., RANDRIAMAMPIONONA S., ANDRIAMANANTENA H., RAZAFIARIMANANA V., RASOAMAMPIONONA R., RAKOTOARISOA N., TSIRINIRINDRAVO H.L., ANDRIANJAKA A., DUPONNOIS R., ANDRIANARISOA B. « Effets des produits volcaniques sur les cultures du riz et maïs et dynamique de la microflore tellurique sur la culture de maïs » : GDRI-BDDM et les questions de biodiversité 3 au 5 novembre 2010, Lyon-France.

[26.] TAMBEKARAND D.H., MUNDHADA R.H. Bacteriological quality of salad vegetables sold in Amravati City (India), Journal of Biological Sciences, vol.6, no.1, pp.28–30, 2006.

[27.] TINKER I. (1997).Street Foods, Urban Food and Employment in developing Countries. Oxford. University Press, New York, 1997. pp 124-126

[28.] TOMOYASU, T., YAMANAKA, K., MURATA, K., SUZAKI, T., BOULOC, P., KATO, A., NIKI, H., HIRAGA, S., OGURA, T. 1993. Topology and subcellular localization of FtsH protein in *Escherichia coli*. J. Bacteriol. 175, 1352–1357.

[29.] TSIRINIRINDRAVO H. L., RANDRIANIERENANA L. A., ANDRIANARISOA B., RAHERIMANDIMBY M., RANDRIANANTOANDRO H. H., RAZAFINJATOVO D. N., DE PERCIN G., PIERLUIGI B. *Aspects épidémio-cliniques des toxi-infections alimentaires collectives (TIAC) dans la région Analamanga, cas de janvier à juin 2015. Communication lors des Journées QualiReg 2016. 5eme rencontre de l'Agroalimentaire en Océan Indien « La qualité et l'innovation au service du développement des filières agroalimentaire de l'Océan Indien. Saint Pierre, La Réunion.*

[30.] TSIRINIRINDRAVO H.L., ANDRIANARISOA B. Société Française de Microbiologie VIIIe Congrès National Marseille 2010, carrefour des microbiologies du Nord et du Sud Palais des Congrès, parc Chanot, Marseille SFM MARSEILLE 2010 - ABSTRACT n°039 Programme : ACTIVITES ANTIBACTERIENNES DE L'EXTRAIT ISSU DE MESANTHEMUM RUTENBERGIANUM (ERIOCAULACEES). 2, 3 et 4 juin 2010.

[31.] TSIRINIRINDRAVO H. L., RANDRIANIERENANA L. A., ANDRIANARISOA B., RAHERIMANDIMBY M., RANDRIANANTOANDRO H. H., RAZAFINJATOVO D. N., DE PERCIN G., PIERLUIGI B.Espèces de *Salmonella*multiresistantes véhiculées par les aliments de rue à Antananarivo. *Communication lors des Journées QualiReg 2016. 5eme rencontre de l'Agroalimentaire en Océan Indien « La qualité et l'innovation au service du développement des filières agroalimentaire de l'Océan Indien. Saint Pierre, La Réunion.*

[32.] TSIRINIRINDRAVO H.L., ANDRIANARISOA B. Activités antibactériennes de l'extrait des feuilles de *Dalechampiaclematidifolia* (Euphorbiaceae). Int. J. Biol. Chem. Sci. 3(5): 1198-1202, October 2009.

[33.] WATARU H., NORIYUKI N. High prevalence of blaCTX-M-14 among genetically diverse *Escherichia coli* recovered from retail raw chicken meat portions in Japan. Journal of Microbiology. August 2018, Volume 56, Issue 8, pp 530–535

[34.] WORLD HEALTH ORGANIZATION, Food borne diseases; a focus for health education, 53rd world health assembly, Geneva, 2000.

[35.] WORLD HEALTH ORGANISATION (1984). The Role of Food Safety in Health and Development. Technical Report Series 705. Expert Committee on Food Safety, Geneva: WHO: 1-79.