International Journal of Innovation Engineering and Science Research
(ISSN: 2581-4591)
www.ijiesr.com

Antimicrobial susceptibility of isolated strains from selected street foods in Antananarivo, Madagascar from January 2017 to December 2017

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ABSTRACT

Street food consists of ready-to-eat foods or drinks sold by a vendor, in a street or other public place. Consumers rely on the quick access and cheap service of street food for daily nutrition. However, these foods carry a large number of microorganisms. Among these microorganisms, there are pathogens, toxinogens, resistant organisms. The aim of this study was to examine bacterial profile, bacterial load, and antimicrobial susceptibility of bacterial isolates among street vended foods in Antananarivo, capital of Madagascar.

A total of 72 food samples from four different food items were analyzed and counted by standard aerobic plate count method.

Two hundred twenty one (253) samples including 102 samples of melting salads, 18 beef skewers, 15 chicken skewers, and typical Malagasy foods as : mofoanana (12 samples), mofogasy (10 samples), ramanonaka (10), makasaoka (14), mofoakondro (18) and kobandravina (22) ; were randomly collected from the street vendors in Antananarivo markets to evaluate their bacteriological quality. International Methods (ISO) was adopted for to find the load of Total Aerobic Bacteria and Enterobateriaceae, Escherichia coli and to search pathogen bacteria as Salmonella, Campylobacter jejuni, Escherichia coli O157H7 and Bacillus cereus in these foods.

Antibiotic susceptibility testing was done for pathogen isolated species, using Muller Hinton agar and data was entered and analyzed by XLSTAT.

The results revealed that the mean values ofthe Total Aerobic Bacteria count was $0.1x10^6$-4.8x106cfu/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 (β glucuronidase -) were isolated.

Among the bacteria strains isolated, *Staphylococcus aureus, Salmonella* and *E coli* were the most resistant. The other isolated bacteria were all susceptible. A high resistance to Ampicillin and Gentamycin was noted compared to the isolated organisms. Trimethoprim, Sulfamethoxazole, Norfloxacin, Ciprofloxacin, Chloramphenicol were found to be the most effective antimicrobials against all strains.

Keywords: street foods, *Escherichia coli, food borne diseases, Antananarivo, Madagascar, antibacterial resistance.*

I. INTRODUCTION

The World Health Organization defines street food as foods and beverages prepared and sold by vendors in streets and other public places for immediate consumption (34).

The street food is a growing sector in many developing countries. They provide a source of very cheap affordable meal, while providing a source of income for the vendors.

Anyway, street vended food products may represent a risk due to a lack of basic infrastructure such as water connections and refrigeration, inadequate personnel hygiene of vendors, using raw materials of bad quality, hygienic practices utilized during transport of products to the vending area. Such contamination may render the product of inferior quality or unfit for human consumption (3).

It has been shown that Street-vended foods have been concerned in outbreaks of foodborne illnesses all around the world. In Madagascar, there was three food borne diseases due to *Salmonella typhi* reported on 2015-2016 and *Escherichia coli* was identified as responsible of so many infections and toxi-infections on 2017 (29).

Pathogenic microorganisms such as *Escherichia coli, Salmonella* and *Campylobacter jejuni* have recognized to be responsible for 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 2017 to December 2017, and to assess the resistance of isolated microorganisms to antibiotics.

II. MATERIAL AND METHODS

Collection of samples

Two hundred twenty one (253) samples including 102 samples of melting salads, 18 beef skewers, 15 chicken skewers, and typical Malagasy foods as : mofoanana (12 samples), mofogasy (10 samples), ramanonaka (10), makasaoka (14), mofoakondro (18) and kobandravina (22) ; were randomly collected from the street vendors in Antananarivo markets. Samples were sent to the laboratory within two hours after collection in a cold-box containing ice-blocks.

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-Vasilliadis Soya broth (RVS). These were incubated at 41^{o}C for 24 h. The culture was streaked into Hektoen 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 ISO 16649 (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* O157 H7 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).

Antibiotic sensitivity testing or **antibiotic susceptibility testing**

Bacteria on the selective media were isolated, purified and identified. The antibiotic resistance of the identified pure strains was carried out.

The growth method was performed. At least three well isolated colonies of the same morphological type was selected from an agar plate culture. The top of each was touched with a loop, and the growth was transferred into a tube containing 4 to 5ml of a suitable broth medium. The broth culture was incubated until it achieved or exceeded the turbidity of the 0.5MacFarland standards which took up to six hours.

Inoculation of test plates.

Optimally, within 15 min after adjusting the turbidity of the inoculum's suspension, a sterile cotton swab was dipped into the suspension. The swab was then rotated several times and pressed firmly on the inside wall of the tube above fluid level.

Excess inoculums were removed from the swab. The dried surface of a mueller-hinton agar was inoculated by streaking the swab over the entire sterile agar surface. this procedure was repeated by streaking two or more times, by rotating the plate to ensure an even distribution of inoculums.

The rim of the agar was swabbed. The lid was left ajar for some minutes to allow for any excess surface moisture to be absorbed before applying the drug-impregnated discs.

Placement of discs

The predetermined battery of antimicrobial discs (Chloramphenicol, Gentamycin, Ciprofloxacin, Trimethoprim, Sulfamethoxazole, Ampicilline, Norfloxacin) was dispensed onto the surface of the inoculated agar plate. Each disc was pressed down to ensure complete contact with the agar surface.

The plates were then inverted and placed in an incubator within 15 minutes after the discs are applied.

Reading plates and interpreting results

After 16 to 18 hours of incubation at 37°C, each plate was examined, the diameters of the zones of inhibition are measured, including the diameter of the disc.

The sizes of the zone of inhibition are interpreted by referring to the (SFM, WHO) table on zone diameter interpretive standards.

III. 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 (>10^6 ufc/g), Enterobacteriaceae (>10^2/g) and *Escherichia coli* β glucuronidase + is noted.

The values of the 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 and *Escherichia coli* count range from 0.04x10^2 cfu/g. to 0.19 x10^2 cfu/g.

Pathogen bacteria as *Salmonella* was only present in melting salads, beef skewers, 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 beef and chicken skewers. Two strains of *Eschercichia coli* O157 H7 (βglucuronidase -) were isolated and identified by PCR reaction from beef skewers.

Two hundred twenty one (253) samples including 102 samples of melting salads, 18 beef skewers, 15 chicken skewers, and typical Malagasy foods as : mofoanana (12 samples), mofogasy (10 samples), ramanonaka (10), makasaoka (14), mofoakondro (18) and kobandravina (22).

Table 1: Microbiological assessment of street foods samples collected in Antananarivo market on 2017-2018.

Number	Samples	TAB.10^6/g	Ent. 10^2/g	E.C.BG+ 10^2/g	E.C.BG-/g	SLM/g	CAMP/g	BC10^2/g
102	Melting salads	5,77	2,61	1,34	A	9,52	A	2,01
18	Beef skewers	4,52	1,98	1,01	2	14,40	0,19	A
12	Mofoanana	0,61	0,97	0,10	A	A	A	0,47
14	Makasoka	0,14	0,36	0,07	A	A	A	0,19
10	Mofogasy	0,70	0,88	0,23	A	A	A	0,18
10	Ramanonaka	0,68	0,54	0,31	A	A	A	0,16

18	Mofoakondro	0,31	0,555	0,067	A	A	A	0,374
54	Chicken skewers	3,998	1,961	1,338	A	2,06	0,942	A
15	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

In this study, usually used antibiotics are selected for the antibiotic susceptibility testing. The antibiotic selection also depends on the bacterial species because different bacterial species need different classes of antibiotics for optimal antibacterial activity. Some of the antibiotics were not tested in this study because some bacterial species are naturally resistant to certain classes of antibiotics; hence the antibiotics were excluded from the analysis.The bacterial isolates and their percentage of resistant are shown in Table 2.

Table 2 : Percentage of resistant of bacterial isolates

Antibiotic susceptibility patterns of isolates		Salm	%	B c	%	*E coli*	%	*Ec* O157	%	*Ca. j*	%	*S.aur*	%	Sty%
chloramphenicol	S	24	96,00	12	100,00	37	84,09	2	100,00	1	100,00	51	82,26	93 ,72
	R	1	4,00	0	0,00	7	15,91	0	0,00	0	0,00	11	17,74	
Gentamycin	S	4	16,00	11	91,67	42	95,45	-	-	1	100,00	43	69,35	74,49
	R	21	84,00	1	8,33	2	4,55	-	-	0	0,00	19	30,65	
ciprofloxacin	S	23	92,00	12	100,00	39	88,64	2	100,00	1	100,00	52	83,87	94,08
	R	2	8,00	0	0,00	5	11,36	0	0,00	0	0,00	10	16,13	
trimethoprim	S	25	100,00	-	-	40	90,91	2	100,00	-	-	-	-	97,72
	R	0	0,00	-	-	4	9,09	0	0,00	-	-	-	-	
Sulfamethoxazole	S	22	88,00	12	100,00	38	86,36	2	100,00	1	100,00	57	91,94	94,38
	R	3	12,00	0	0,00	6	13,64	0	0,00	0	0,00	5	8,06	
Ampicilline	S	12	48,00	11	91,67	31	70,45	-	-	1	100,00	10	16,13	60,89
	R	13	52,00	1	8,33	13	29,55	-	-	0	0,00	52	83,87	
Norfloxacin	S	25	100,00	12	100,00	34	77,27	2	100,00	1	100,00	54	87,10	94,06
	R	0	0,00	0	0,00	10	22,73	0	0,00	0	0,00	8	12,90	
Rce%			22,85		2,38		15,00		0,00		0,00		24,19	

Salm : *Salmonella*, B.c. : *Bacillus cereus*, E coli : *Escherichia coli*, Ec O157 *Escherichia coli* O157H7, Ca.j : *Campylobacter jejuni*, S. aur : *Staphylococcus aureus*,S : Sensitive, R : resistant, Sty : susceptibility, Rce : resistance

The most resistant are: *Staphylococcus aureus*, especially against Beta-lactam antibiotics (Ampicillin). The percentage resistant is around 83.87%. Then there is *Salmonella*, resistant to Ampicillin (52.00%) and Gentamycin, in the order of 84.00%. In third place, there is *Eschercichia coli* with a resistance of 29.55% against Ampicillin and 22.73% against Norfloxacin.

Staphylococcus aureus is naturally susceptible to virtually every antibiotic that has ever been developed. Resistance is often acquired by horizontal transfer to genes from outside sources, although chromosomal mutation and antibiotic selection are also important.

Salmonella is a main pathogen in humans as well as in animals and comprises 12000 serotypes. They are generally dispersed in nature and are common inhabitants of the intestinal tract of domesticated and wild mammals, reptiles, birds, and even insects.
Increasing antimicrobial resistance in *Salmonella* species has been a serious problem for public health worldwide. The high percentage of resistance is hampering the use of conventional antibiotics, and growing resistance to newer antimicrobial agents is aggravating the situation. The circumstances of occurrence and spread of antimicrobial resistance are complex; however, a main cause is the widespread use of antimicrobial agents in food animals, particularly in animal feed. Genetic analysis has shown that the source of resistance is frequently a transferable plasmid. Current studies have revealed that some serotype-specific virulence plasmids form hybrid plasmids through recombination with resistance plasmids or acquire gene cassettes consisting of multiple resistance genes. Such evolutionary events provide a virulent strain the advantage of survival in an unfavorable drug

environment. In view of the serious implications associated with drug-resistant *Salmonella* species, a more careful use of antibiotics in both human medicine and animal industry is warranted. Continued surveillance of antimicrobial resistance and use of antimicrobial agents in food animals is also indispensable.

Patients with invasive salmonellosis require antimicrobial treatment. Increasing antimicrobial resistance may add to the difficulty or delay in administration of microbiologically effective therapy, leading to increased morbidity and mortality. On the other hand, antimicrobial use causes a transient decrease in an individual's resistance to colonization with noncommensal bacteria and increases the probability of infection on exposure to a foodborne pathogen, such as *Salmonella* species.

The statistical analysis compared to the antibiotics tested shows that the bacterial isolates have highest percentages of susceptible were towards Trimethoprim (97.72%), Sulfamethoxazole(94.38%), Ciprofloxacin, followed by Chloramphenicol 93.72% and Gentamycin which is around 74.49%.

The bacterial isolates showing high percentages of resistant were towards Ampicillin, (60.89%), which may affect the treatment of diseases caused by these germs. We can see a therapeutic failure.

Still according to these results, pathogenic germs therefore circulate in community settings, in extra-hospital settings. Foodstuffs are the carriers of these germs.

The presence of these bacteria could be due to the use of phytosanitary products, antibiotics or feed that contain a high amount of antibiotics,...

In this study, the antibiotic resistant patterns for all isolates were also determined to monitor the spread of antibiotic resistance. There is species isolates with 0% resistance towards all antibiotics tested in all sampling: *Escherichia coli* O157H7 and *Campylobacter jejuni*.

IV. Discussions

Isolation of antibiotic resistant bacteria from street foods indicates the health risk associated with these type of food.

There had been reports on detection of antibiotic resistance genes in bacteria isolated that can be transferred to human microbiota.

In antibiotic resistance analysis, the history of antibiotic application in particular area is reflected by the percentage of bacterial resistance to antibiotics. The frequency of antibiotics usage is related to the level of resistance among bacteria. In this present study, high percentage of susceptibility was observed towards Trimethoprim (97.72%), Sulfamethoxazole (94.38%), Ciprofloxacin, followed by Chloramphenicol 93.72% and Gentamycin which is around 74.49%.

Gentamicin was approved for use in 1963 (*2*). Although gentamicin resistance was rare in human *E. coli* isolates, we found resistance rates <40% among animal *E. coli* in 2002. Since 1980, resistance to gentamicin has increased among animal *E. coli* isolates.

Similarly, Tsirinirindravo et al. in their studies observed a high percentage of sensitivity of Gram negative bacteria (*Escherichia coli, Salmonella typhi)* to this family of antibiotics, especially Trimethoprim family and Ciprofloxacin *(second-generationquinolones) (31)*.

High percentage of Ampicillin resistance was observed in this study. Similarly, high ampicillin and streptomycin resistance were also observed by Zhang et al. in their study on antibiotic resistance detection in *E. coli* strains isolated from communautary area in South China (26).

Antibiotic resistance pattern may vary depending on the geographical locations and selective pressure and these patterns change rapidly from time to time. The different patterns exhibited by different strains or species suggest how complex is the understanding of the antibiotics resistance in the study area.

It is expected that environments where antibiotic use is high will select for a high level of antibiotic resistance in isolated bacteria. Therefore, the higher number of multidrug resistant bacteria from the rehabilitation centres was not surprising.

Awareness on antibiotic resistance threat should be instilled in the community regardless of age as precaution and prevention step against dissemination of antibiotic resistant bacteria.

The community must be educated on antibiotics and their effects on public health. Many surveillance programs had also been introduced to monitor the emergence and spread of antibiotic resistant bacteria. Plasmid screening should be considered as an additional procedure in the monitoring programs to trace antibiotic resistance dissemination.

Conclusion

The study aims to determine the microbial quality and the sensitivity of bacteria isolated frompre-cut ready-to-eat vegetable salads sold by food vendors in the Antananarivo markets on 2017.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.

Staphylococcus aureus, Salmonella and *E coli* were the most resistantcompared to the antibiotics tested. A high resistance to Ampicillin and Gentamycin was noted compared to the isolated organisms.
Trimethoprim, Sulfamethoxazole, Norfloxacin, Ciprofloxacin, Chloramphenicol were found to be the most effective antimicrobials against all strains.
The contamination could come from unhygienic food preparation, process, environmental conditions, raw materials and improper food handling. The circumstances of occurrence and spread of antimicrobial resistance are complex; however, a main cause is the widespread use of antimicrobial agents in food animals, particularly in animal feed.

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