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Assessment of the microbiological quality of beverages sold in collective cafes on the campuses of the University of Abomey-Calavi, Benin Republic

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ABSTRACT

Fresh juices are highly nutritious foods for human beings, but the inability to observe requirements for their preparation, packaging and storage subjects them to microbial contamination which poses a potential health risk to consumers. The purpose of this study was to evaluate the microbiological quality of beverages sold within the cafes of the campuses of Abomey-Calavi University (Benin). A survey carried out among beverage vendors showed that the sources of contamination were uncontrolled and the raw materials used were of questionable quality as the operators lacked good hygienic practices. Thus, the microbial quality of forty-five samples of four types of beverages sold in these cafes was investigated for mesophilic aerobic flora, *Coliforms*, *Escherichia coli*, *Staphylococcus aureus*, sulfate-reducing anaerobic spores, fungal flora and *Salmonella spp*. using standardized methods. Then, molecular studies identified the pathogenic strains isolated from the beverages. An antibiotic susceptibility test was performed on the strains identified for the detection of multi-resistant bacteria. These analyses revealed a non-compliance rate of 100% in the analyzed samples. The indicators that caused this non-compliance in the samples were mesophilic aerobic flora, coliforms and fungi. In addition, 85.7% of the samples contained other *Enterobacteriaceae* including *Klebsiella pneumoniae*, *Morganella morganii*, *Kluyvera georgiana*, *Citrobacter murliniae, Yersinia intermedia*. While the non-compliance rates of the samples for *Salmonella spp* and *E.coli* were 4.4% each, the non-compliance rate for *S. aureus* was 2.2% with the presence of sometimes multi-resistant pathogenic bacteria. Sellers' awareness of good hygiene practices is important for improving the quality of food sold.

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1. Introduction

The rapid urbanization of developing countries and the multiple daily constraints faced by urban dwellers have facilitated the emergence of new food consumption patterns (1). An increasing number of people rely on street foods for their daily diet (2), which enable about 80% of the urban population (pupils, students, employees, unemployed, street children and shopkeepers) to easily take meals outside the home and at low cost (3).

At present, the street food sector is undoubtedly one of the most important sectors of activity providing urban employment in developing countries. These incomes allow several households to cover the family's financial needs (4). However, street foods are perceived as a major public health risk due to the lack of basic infrastructure, difficulties in controlling the large number of meals served, their diversity and nature (5).

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Also, most of these foods are not covered and are exposed to flies and dust that can harbour pathogens (6). Many studies have reported the role of street foods as vectors of pathogenic bacterial transmission to humans (7). Thus, foodborne diseases are an important cause of morbidity and mortality because millions of people fall ill and many die after ingesting food unfit for consumption (8). They are caused by several microorganisms including *Staphylococcus aureus, Bacillus cereus, Clostridium perfringens, Salmonella spp* and pathogenic *Escherichia coli,* which cause various consumer disorders. Indeed, *Staphylococcus aureus*, through the production of enterotoxins, is responsible for food poisoning manifested by the following clinical signs: vomiting, diarrhoea, abdominal pain, nausea sometimes accompanied by headache and neurological disorders (9). *Clostridium perfringens* intoxications are characterized by acute abdominal pain, diarrhoea, nausea, fever and sometimes vomiting (10).

Salmonella has a pathogenic feature due to its entero-invasive capacity, which is manifested by salmonellosis characterized by anorexia, headache, chills, fever, nausea, vomiting, abdominal pain and diarrhoea (11). *Escherichia coli* can also cause intestinal infections in some cases. Some of its strains may be enteropathogenic, enterotoxigenic, entero-invasive or enterohaemorrhagic, which may be manifested by diarrhoea nausea, vomiting, abdominal pain, fevers, renal failures, thrombocytopenia etc. (12). These various diseases are related to the consumption of many foods, including drinks sold in collective catering and contaminated by certain bacteria or their toxins (13). On the campuses of the University of Abomey-Calavi, food cafes have considerably increased with the evolution of the population attending the campuses. A study conducted by Ahoyo et al (15) on the safety of food sold on Abomey-Calavi university campuses and the risks of foodborne diseases among consumers revealed that 74% of samples were contaminated with some pathogenic microorganisms such as *Escherichia coli, Salmonella spp., Staphylococcus aureus* and *Enterococcus spp.* This work, which is an update of the previous one, was carried out in order to assess the microbiological quality of beverages sold in campuses of University of Abomey-Calavi and their safety for human's consumption in terms of pathogenic bacteria.

2. Materials and methods

The material used for this study consists of samples of bissap juice, pineapple juice, "Akpan", a yoghurt-like cereal product and "Dèguè", a fermented milk mixed with pellets collected in the shopping centres (sites) of the three campuses: Calavi campus, National School of Applied Economics and Management (ENEAM) and Faculty in Health Sciences (FSS) campuses. This study was conducted in three phases: investigation, sampling and laboratory analysis.

2.1. Investigation

A survey was conducted in the form of interviews with liquid food vendors at the various study sites, supported by a questionnaire. A total of 55 people were surveyed. The various information collected is summarized under 5 main headings: raw material, material, labour, method and environment. The inventory of food preparation and sale was carried out according to points listed in fact sheets prepared for the study.

2.2. Microbiological analysis

The forty-five beverage samples used in this work were purchased in their containers of vendors from the campuses of the University of Abomey-Calavi (Calavi Campus; ENEAM and FSS at the Cotonou University Centre) from different categories of vendors. The beverages were selected on the basis of the degree of potential risk they may pose, their contamination and their high frequency of consumption, based on the SHSA report (15). The material consists of 45 drink samples: 15 Bissap juice, 15 pineapple juice, 12 "Akpan", 03 "Dèguè".

2.2.1. Microorganisms enumeration

Several microorganisms summarized in Table 1 were counted or detected according to standard methods (16, 23).

Table 1: Used microbiological methods

Parameters	Media	Standard methods used
Mesophilic Aerobic Flora at 30°C	Plate Count Agar (PCA OXOID CM0463)	NF EN ISO 4833-2 : 2013
Total coliforms at 30°C	Brillance *E. coli* OXOID CM01046	Modified NF ISO 4831 : 2006
Fecal coliforms at 44°C	Brillance *E. coli* OXOID CM01046	Modified NF V08-060 : 2009
Sulphite reducing anaerobic bacteria	Trypticase-Sulfite-Neomycin Agar (TSN Biokar 001HA)	NF V 08-061: 2009
Coagulase positive Staphylococcus	Baird Parker (BP OXOID CM09761) + RPF	NF EN ISO 6888-3 : 2003
***Salmonella* spp**	- EPT OXOID CM0509 - Rappaport-Vassiladis (OXOID CM0669) - Hektoen (OXOID CM0419) - XLD (OXOID CM0469)	Modified NF EN ISO 6579-1 : 2017
Yeast and moulds	Sabouraud Dextrose agar (OXOID CM 0041)	NF ISO 21527-1 : 2008 and NF ISO 21527-2 : 2008

2.2.2. Biochemical identification

This step consisted in highlighting the different strains by means of the specific biochemical tests API 20 E gallery, the API Staph gallery and the OXOID 12 S gallery.

2.2.3. Molecular identification

Some microorganisms namely *Escherichia coli*, *Klebsiella pneumoniae, Salmonella spp* and *S. aureus* that were identified by biochemical tests have been confirmed at the molecular level using 16 S universal PCR and sequencing.

2.2.4. DNA extraction

Extraction of DNA was carried out by thermal shock using the method reported by Somda et al (24), which consists of boiling the microbial suspension at 100°C for 10 min and then collecting the supernatant after centrifugation. The genomic DNA concentration of the samples was determined using a Nanodrop 2000 Thermo Scientific ISOGEN spectrophotometer. Universal PCR 16S. PCR amplification of the previously extracted DNA was performed in a 20 µl reaction containing 2 µl of 10 x PCR buffer; 2 µl of dNTP (2 mM); 1.6 µl of $MgCl_2$ (25 mM); 0.2 µl of the Taq polymerase (5 U/µl) and 0.8 µl of each of the two (02) primers (5' AGAGTTTGATCCTGGCTGCTCAG3' and 5' CGGCTACCTTGTTTTACTT3' reverse primer); 10.6 µl PCR quality water and 2 µl DNA sample were added. The cycling conditions used in the thermal cycler were 94°C for 5 min for the initial denaturation step followed by 35 denaturation cycles at 94°C for 30 s, then hybridisation at 56°C for 30 s and elongation at 72°C for one min with a final extension at 72°C for 5 m. These steps were followed by a storage phase at 4°C. The migration was done on 0.8% agarose gel at 100 Volts for 30 m and the visualization of the fragments obtained after migration was done with the Gel Doc EZ Imager BIO-RAD. PCR products were sequenced in Laboratory GIGA at University of Liege, Belgium after a purification step (25) using the Kit Wizard. An equimolar mixture of each product and the direction primer was used for sequencing.

2.2.5. Antibiotic susceptibility of pathogenic strains

The susceptibility of isolates to several antibiotics (Table 2) was tested by the Mueller Hinton agar diffusion method according to the recommendations of CLSI (Clinical and Laboratory Standards Institute) (26,27) and EUCAST (European Committee on Antimicrobial Susceptibility Testing) (28). After 24 h of incubation, the inhibition diameters were measured using the caliper and the susceptibility of the isolates to antimicrobial agents was assessed after comparing the values obtained with critical values (CLSI and EUCAST). Thus, an isolate may be susceptible (S) to an antimicrobial agent or intermediate (I) or resistant (R).

Table 2: Antibiotics tested and their classes

Antibiotics	Classes
Ampicillin (AMP) 10 µg	Beta lactamine Penicillin group A
Amoxicilline-clavulamic acid (AMC) 20/10 µg	Beta lactamine Penicillin group A
Aztreonam (ATM) 30 µg	Beta lactamine Monobactam
Cephalotin (CEF) 30 µg	Beta lactamine Cephalosporin 1st generation
Cefoxitin (FOX) 30 µg	Beta lactamine Cephalosporin 2nd generation
Cefotaxime (CTX) 30 µg	Beta lactamine Cephalosporin 3rd generation
Chloramphenicol (CHL) 30 µg	Phenocolate
Ciprofloxacin (CIP) 5µg	Quinolone
Gentamicin (GMN) 10 µg	Aminoside
Sulfonamide (SSS 300) 300 µg	Sulfamides
Tetracycline (TET) 30 µg	Tetracycline

2.2.6. Statistical analysis of data

The collected survey data was analyzed by first carrying out a cross-sectional description to ascertain the health and safety conditions of beverages sold on University of Abomey-Calavi campuses. A frequency of each of the variables characterizing the raw materials, the methodology of work, the man power, the material of work and the environment to determine the level of importance of each variable was calculated. In order to compare the different targeted microbiological parameters of foods against the average values of the parameters considered, a descriptive analysis was carried out. Thus, to compare the variation in the rate of the microbiological parameters considered from one seller to another, an analysis of variance (ANOVA) was carried out. Also, in order to comply with the conditions for the application of analysis of variance, logarithmic transformation was performed for data on mesophilic aerobic flora, total coliforms, faecal coliforms, yeasts and staphylococci. In addition, the Tukey test was carried out to structure the averages. The same analyses were done on antibiotic inhibition diameters. All analyses were performed with the R 3.4.3 software.

3. Results

3.1. Investigation

The study included a total of 55 vendors offering the following products: pineapple, papaya, bissap, lemon, tamarind, "adoyo", "akpan", "dèguè", tapioca juice. A proportion analysis of the collected data was done in the R software. The results are presented in tables (3-7).

3.1.1. Raw materials

In table 3, analysis of the data collected showed that 50.91% of sellers take into account the price for the purchase of raw materials, while 1.82% buy them according to the cleanliness of the selling establishment and 32.7% do not protect food. In general, raw materials come from various sources (85.45%).

Table 3: Raw material and ingredient characteristics

Variable	Modalities	Frequencies %	IC
Criteria for choosing raw material suppliers	Prices	50.91	13.21
	Proximity to the point of sale	34.55	12.57
	Cleanliness of the establishment	1.82	3.53
	Cleanliness of the establishment, Price	12.73	8.81
Source of supply of raw materials	Unique	14.55	9.32
	Various	85.45	9.32
Protection of food sold	Yes	67.27	12.40
	No	32.73b	12.40

Legend: CI: Confidence Index

3.1.2. Hygiene habits of staff

The employees were not subjected to medical checks. Jewellery is removed (74.55%) and the percentage of actors with uncut, uncleansed and varnished nails is highly significant (36.36%). Vendors generally handled money during the service (94.55%) and hand washing before food service was rarely done (Table 4).

Table 4: Staff Characteristics

Item/Variable	Modality	Frequencies %	IC
Medical notebook	Yes	60	12.43
	No	40	10.95
Updated medical notebook	Yes	60	12.43
	No	40	10.95
Training on good hygienic practices	Trained	25.45	11.51
	Not Trained	74.55	11.51
Wearing the appropriate uniform	Yes	21.82	8.59
	No	78.18	13.08
Apparent cleanliness of the uniform / clothing	Yes	78.18	13.08
	No	21.82	8.59
Hair protection	Yes	47.27	11.59
	No	52.73	11.99
Use of jewellery	Yes	25.45	9.17
	No	74.55	13.00
Clean, short, unpainted nails	Yes	63.64	12.71
	No	36.36	12.71
Hand washing before serving food	Yes	29.09	12.00
	No	70.91	12.00
Handling money during the activity	Yes	94.55	6.00
	No	5.45	6.00

Legend: CI: Confidence Index

3.1.3. Working material

The equipment used to serve drinks consists of plastic bowls and cans (72.73%) and sometimes aluminium bowls (10.91%). The large number (90.91%) sold outdoors, under trees and parasols. Most utensils were clean, but a significant number used dirty utensils (23.63%).

Table 5: Characteristics of the work equipment

Item/Variable	Modalities	Frequencies%	IC
Disposal of a room	Available for sale	90.91	7.60
	Not-available	9.09	7.60
Water supply source	Campus	43.64	13.11
	Home or elsewhere	16.36	9.78
	Campus, Home or elsewhere	40	12.95
Condition of the utensils	Clean	76.36	11.23
	Dirty	23.63	11.23
Nature of utensils used to serve food	Aluminium	10.91	8.24
	Plastic	72.73	11.77
	Plastic, Aluminium	16.36	9.78

Legend: CI: Confidence Index

3.1.4. Methodology of work

Almost half of the actors do not have a refrigerator for storing drinks according to observations. The latter are therefore not served as a cold link. Sterilization of spoons by heating during food service is not carried out by 40% of the respondents and cleaning is insufficient and is carried out with disinfectants (Table 6).

Table 6: Characteristics of the working method

Item/Variable	Modality	Frequency %	IC
Separate utensils for each food or preparation	Regular	10.91	8.24
	Irregular	67.27	12.40
	Never	21.82	10.92
Cold link service for cold food	Yes	45.45	13.16
	No	54.55	13.16
Food category	Hot Foods	7.27	6.86
	Cold foods	92.73	6.86
Preparation day	Day of sale	18.18	10.19
	Watchfulness	50.91	13.21
	Sale day, vigil	30.91	12.21
Heating of utensils during service	Heating	60	12.95
	Doesn't heat	40	12.95
Proper storage and maintenance of food	Store	58.18	13.04
	Do not store	41.82	13.04

Legend: CI: Confidence Index.

3.1.5. Point of sale

The survey revealed an unhealthy sales area with sometimes garbage near the sales stands. The respondents did not have adequate lighting (9.09%) at the production sites. 20% of respondents didn't have appropriate location for sales and were not subjected to any controls (Table 7).

Table 7: Characteristics of the beverage sales and manufacturing environment

Item/Variable	Modality	Frequency (%)	IC
Environment	Clean	63.64a	12.71
	Dirty	36.36b	12.71
Sanitary installation	Satisfactory	69.09a	12.21
	Unsatisfactory	30.91b	12.21
Place of Preparation	House	54.55a	13.16
	On the spot	45.45a	13.16
Lighting	Sufficient	90.91a	7.60
	Insufficient	9.09b	7.60
Location	Appropriate	80a	10.57
	Not appropriate	20b	10.57

Legend: CI: Confidence Index; The frequencies for each item followed by different letters are significantly different at the 5% threshold

3.2. Microbiological quality of the beverages and isolate-identification

All 45 beverage samples were contaminated with mesophilic aerobic flora which values were ranged from 3.41±2.77 log cfu/ml to 8.82±8.81 log cfu/ml for pasteurized beverages and from 6.78±6.5 log 10 cfu/ml to 8.99±8.99 log 10 cfu/ml for unpasteurized drinks (Table 8). 4.44% and 22.22% of the beverage samples were respectively contaminated by presumptive *E. coli* and other *Enterobacteriaceae* mainly dominated by *Klebsiella pneumoniae*. The presence of pathogenic bacteria was also observed namely *S. aureus* (2.22%), *Salmonella spp* (4.44%), *Klebsiella pneumoniae, Raoultella terrigena, Kluyvera ascorbata, Yersinia intermedia, Citrobacter murliniae, Yokenella regensburgei, Morganella morganii* and *Staphylococci (Staphylococcus sciuri, Staphylococcus intermedius)* using galleries API in all samples except bissap juice samples. Three strains of *S. sciuri* were isolated only from a sample of "Akpan".

3.3. Susceptibility of isolated strains to antibiotics

Several antibiotics from different families were tested and the profile of isolates were determined. The different diameters obtained were compared with the standards (CLSI, EUCAST) to determine whether the strains were resistant, intermediate or sensitive to each of the antibiotics. It was found that aztreonam, chloramphenicol and gentamycine had the greatest effect on 100% of *E. coli* followed by Amoxicillin-Clavulanic acid, Cefoxitin and Ciprofloxacin which were active against 83.3% of the same strain.

All the antibiotics tested were active on all strains of *Salmonella spp* with the exception of ciprofloxacin, for which they have an intermediate sensitivity. Regarding the other *Enterobacteriaceae* namely *Klebsiella pneumoniae, Yersinia intermedia, Kluyvera ascorbata, Raoultella terrigena, Kluyvera ascorbata, Yersinia intermedia, Citrobacter murliniae, Yokenella regensburgei, Morganella morganii,* they (96.3%) were sensitive to Cefoxitin. These other *Enterobacteriaceae* are mainly strains of *Klebsiella pneumoniae* which have, in varying proportions, resistance to betalactams namely ampicillin (85.2%), cefotaxime (14.18%), amoxicillin (3.71%), cefoxitin (3.71%), aztreonam (3.71%); Sulfonamides: sulfonamide (40.74%), Tetracyclines (37.03%) and ciprofloxacin (3.71%) (Figure 3). For *S. aureus,* the most active antibiotic was sulfonamide followed by chloramphenicol, cephalotin, gentamicin and ciprofloxacin. To summarize, some *Enterobacteriaceae* (21.42%) isolated from "Dèguè" (*E. coli* and *Yersinia intermedia* and *Kluyvera ascorbata*), "Akpan" and pineapple (*Klebsiella pneumoniae*) are resistant to at least three antibiotics from different families and with a different mode of action. They are called multidrug-resistant.

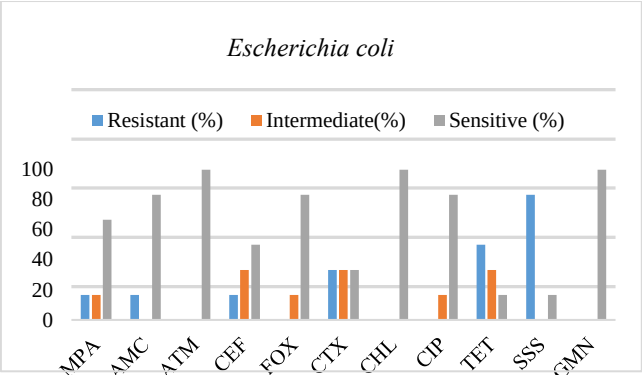

Figure 1: Antibiotic resistance of *E. coli* (n=6) isolated from "Deguè" beverages

MPA : Ampicillin ; AMC : Amoxicillin-Clavulanic acid ; ATM : Aztreonam ; CEF : Cephalotin ; FOX : Cefoxitin ; CTX : Cefotaxime ; CHL : Chloramphenicol ; CIP : Ciprofloxacin ; GMN : Gentamicin ; SSS : SUlfonamide ; TET : Tetracycline

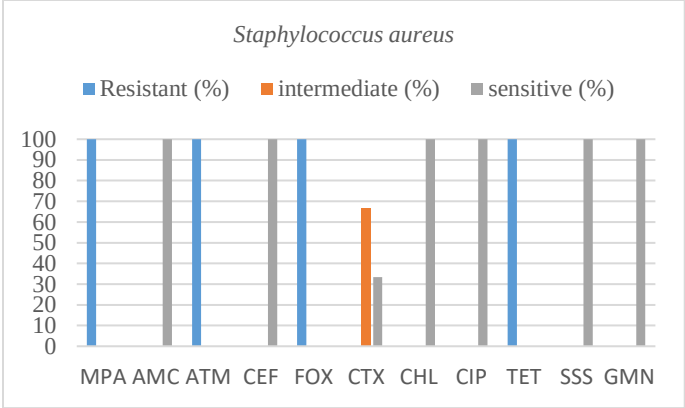

Figure 2: Antibiotic resistance of *Staphylococcus aureus* isolated from beverages (n=3)

MPA : Ampicillin ; AMC : Amoxicillin-Clavulanic acid ; ATM : Aztreonam ; CEF : Cephalotin ; FOX : Cefoxitin ; CTX : Cefotaxime ; CHL : Chloramphenicol ; CIP : Ciprofloxacin ; GMN : Gentamicin ; SSS : SUlfonamide ; TET : Tetracycline

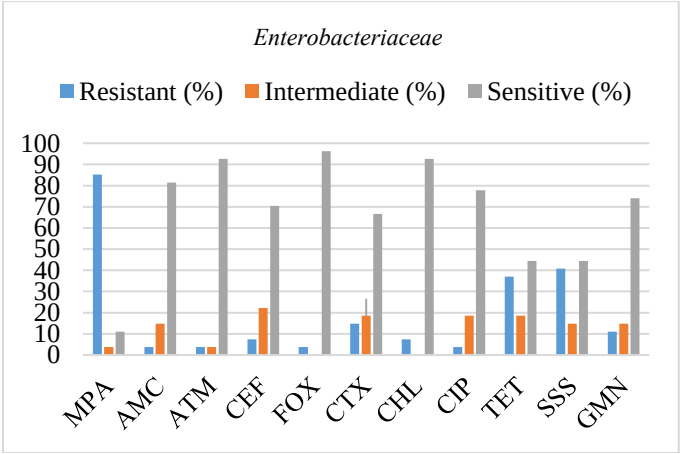

Figure 3: Antibiotic resistance of other *Enterobacteriaceae* isolated from beverages samples (n=27)

MPA : Ampicillin ; AMC : Amoxicillin-Clavulanic acid ; ATM : Aztreonam ; CEF : Cephalotin ; FOX : Cefoxitin ; CTX : Cefotaxime ; CHL : Chloramphenicol ; CIP : Ciprofloxacin ; GMN : Gentamicin ; SSS : SUlfonamide ; TET : Tetracycline

Table 8: Results of microbial analyses of drinks taken from the University of Abomey-Calavi campuses.

	Beverages	MAF (log10 cfu/mL)	Total coliforms (log10 cfu/mL)	Fecal coliforms (log10 cfu/mL)	*E. coli* (log10 cfu/mL)	Yeast (log10 cfu/mL)	Moulds (log10 cfu/mL)	*Coagulase positive Staphylococcus* (log10 cfu/mL)	*Salmonella* spp (Absence in 25ml)	ASR (log10 cfu/mL)
A	Bissap	6.76a	4.47a	3.79a	<1	4.8a	<1	<1	Not detected	<1
D	Bissap	5.99a	4.31a	1.45b	<1	5.19a	<1	<1	Not detected	<1
G	Bissap	4.87b	3.65a	2.88b	<1	3.58b	<1	<1	Not detected	<1
J	Bissap	3.63c	2.47b	<1	<1	2.68b	<1	<1	Not detected	<1
N	Bissap	7.28a	2.84b	1.9b	<1	3.64a	<1	<1	Not detected	<1
B	Ananas	3.41a	1.7a	1.53a	<1	2.77a	<1	<1	Not detected	<1
E	Ananas	8.99c	7.24c	6.99c	<1	5.8c	<1	2.03	2	<1
H	Ananas	6.91b	5.92b	3.77b	<1	4.64b	<1	<1	Not detected	<1
K	Ananas	6.78b	4.92b	2.96b	<1	5.22c	<1	<1	1	<1
M	Ananas	8.82b	4.50b	4.33b	<1	4.92c	<1	<1	Not detected	<1
F	« Akpan »	7.64a	3.67a	2.81a	<1	5.23a	<1	<1	Not detected	<1
I	« Akpan »	7.66b	5.71b	2.37b	<1	6.05b	<1	<1	Not detected	<1
L	« Akpan »	7.77b	4.54b	2.67a	<1	5.91b	<1	<1	Not detected	<1
O	« Akpan »	7.18a	2.87a	2.37b	<1	5.10a	1.89	2.03	Not detected	<1
C	« Dèguè »	8.1	6.74	6.35	3.07	4.27	<1	<1	Not detected	<1
Criteria (log10 cfu/mL)		**4.7***	**2***	**NA**	**< 2****	**3***		**< 4****	**Not detected in 25 mL**	**NA**

Legend: A, D, G, J and N: bissap; B, E, E, H, K and M: pineapple; F, I, L and O: akpan; C: dèguè. MAF: mesophilic aerobic flora; The means followed by the same letter are not significantly different at the 5% threshold and the means followed by different letters are significantly different at the 5% threshold; * Recommended Microbiological standards for any fruit juices sold in the Gulf region (29); **Microbiological Safety criteria of Ready-to-Eat Foods Placed on the Market from Health Protection Agency, London, November 2009 (30); NA: Not applicable

Table 9: Multidrug resistant *Enterobacteriaceae* profile.

	Bêta lactamine						Aminoside	Quinolone	Tetracycline	Phenocolates	Sulfamides
	Penicilin A		Monobactam	Cephalosporin 1st generation	Cephalosporin 2nd generation	Cephalosporin 3rd generation					
	AMP	AMC	ATM	CEF	FOX	CTX	GMN	CIP	TET	CHL	SSS
*E. coli**	R	S	S	S	S	S	S	S	R	S	R
*Y. intermedia**	R	S	S	R	S	R	R	I	I	S	R
*K. ascorbata **	R	S	S	S	S	S	S	S	R	S	R
*K. Georgiana**	R	S	S	I	S	I	S	S	R	S	R
*C. murliniae**	R	S	S	S	S	S	S	S	R	S	R
*K.pneumonia***	R	I	S	S	S	R	S	I	R	R	R
*K.pneumonia****	R	I	S	I	S	I	R	S	R	R	R
*K.pneumonia****	R	S	S	S	S	S	S	S	R	S	R
*K.pneumoniae****	R	S	I	R	S	R	R	R	S	S	R

Legende : R : résistant ; I : intermédiaire et S : sensible * from Degue ; ** from Akpan ; *** : from Ananas

4. Discussion

The present study was conducted on the basis of the quality of raw material used, the working methodology, the hygienic practices followed by employees, the cleanness of material of work and the hygiene of environment of selling and has highlighted problems that are sometimes already known but not always formalized or taken into account in their entirety. This survey revealed that the majority of campus vendors are women (96%) and 74.55% have not received any training in Good Hygienic Practices. The results obtained in this analysis showed that the non-conformities identified during the study may be due to vendors (labour) due to insufficient qualification, competence, training and/or awareness of good hygiene practices and good manufacturing practices in the production, transport, processing and marketing of beverages sold on campus. Street food processing practices in this study showed that 78.18% of vendors served their customers without special clothing and all treated drinks with bare hands. Only 29.1% of vendors wash their hands before serving drinks and this is done with inappropriate ladles, which encourages contact between the drinks and their hands. This percentage is lower than that obtained by Reddi et al (31) who reported that respondents had a good knowledge of food security aspects such as hand washing (92%) in India, as well as those reported by Tshipamba et al (32) in Ethiopia, where 90.9% of people had not used special clothing for food sales and 80.9% had handled food with bare hands. The service of these cold-coupled drinks is not provided by 54.55% of the respondents who serve the ice in the drinks at the time of sale. This food storage condition can encourage the multiplication of the microbial flora found in the food. Therefore, targeted training of vendors on food safety is essential to avoid food contamination by foodborne pathogens.

According to FAO, the risk of food poisoning from street food remains a threat in many parts of the world. As microbiological contamination is one of the major problems, it is recognised that foodborne pathogens represent a serious health risk. The risk depends mainly on the type of food, cooking method and storage. The ignorance or sometime negligence of stakeholders in this sector is a cause of foodborne diseases and a risk factor that cannot be ignored (24). Despite the potential benefits of fruit juices, concerns about their safety and quality have been raised. Some freshly squeezed fruit juices with few or no processing steps to reduce pathogen levels, if contaminated, pose a risk to consumers (33). In this study, pineapple juice and "Dèguè" revealed the presence of high microbial loads consisting of a number of microorganisms such as *E. coli, S. aureus and Salmonella spp.* Research and counting of the total aerobic mesophilic flora in high consumption drinks served in a student environment have yielded unsatisfactory results. The high numbers of this category of microorganims gives an idea of the global contamination according to Mouloudi (34). For

all the beverages studied (100%), the load of aerobic mesophilic germs is above the criteria (4.7 log 10 cfu/ml of beverage) recommended by Gulf standards (29) and reported by Khan et al. (35). This percentage is similar from that found by Sylla and Seydi (36) (98%) in Senegal. The constant and high prevalence of these microorganisms in beverages is probably due to non-compliance with good hygiene practices. Normally, a product with too many total flora is considered unfit for consumption. However, fermented drinks in principle contain a very high number of microorganisms responsible for fermentation, which leads us to recommend more hygiene in the collective catering of the campuses. Knowing that the presence of *Salmonella*, *Staphylococci* and ASR in drinks reflects their insalubrity, 8.88% of the samples are unsatisfactory and can be considered dangerous for consumption. These results are contrary to those of Mouloudi (34) who found no *Salmonella* and *Staphylococci* in food and lower than those of Ahoyo et al (14) who reported that 27% of food sampled in the University of Abomey-Calavi (Benin) was contaminated with *Salmonella* especially *Salmonella enteritidis*. The count of thermotolerant coliforms in beverages showed that 6.67% of samples are satisfactory, contrary to the percentage (16%) reported by Ahoyo et al (14) who detected 21 isolates of *Escherichia coli O157* in similar foods in Benin. A number of factors are responsible for the contamination of freshly squeezed fruit juices. Most fruits contain bacteria reaching 5 log10 cfu/cm^2 on their surface (31). Inappropriate fruit washing adds these bacteria to the juices causing contamination. In addition, the lack of understanding of basic safety issues by suppliers contributes to the increase in microbial loads. In addition, there is often prolonged storage of drinks without refrigeration or with ice produced in an unhygienic way, unhygienic environment with swarming flies and airborne dust (37). A number of studies in different countries have shown that the microbial quality of ice to cool food and beverages may be a concern (38). In fact, according to Awuor et al (39) and Lateef et al. (37), ice presents a potential hazard due to microbiological contamination. The presence of *S. aureus* and *S. sciuri* in pineapple juice can be mainly attributed to the non-pasteurization of this liquid food in view of the particular manufacturing method collected during our investigations. The absence of *S. aureus* in all the samples of bissap juice, "Dèguè" and "Akpan" sampled can be explained by the fact that these drinks undergo a heat treatment during their manufacture. *S. scuiri*, a *Staphylococcus* of animal origin, is an additional indication that suggests the contamination of analyzed foods; because the transmission of these microorganisms is very often caused by human reservoirs (31, 25). We have also observed strains of *Salmonella*, pathogenic bacteria that cause food poisoning, as it is also the case with *S. aureus*. These contaminations can result from poor food preparation practices or faecal contamination when handled by dirty hands. However, the presence of faecal coliforms and *E. coli* in the samples indicates poor hygiene in processing, which may result from the processor, the equipment in contact and/or the immediate environment of the product. These microorganisms indicate not only poor hygienic quality of these drinks, but also a high risk of contracting foodborne infections. The lack of sanitary conditions at the point of sale and the presence of pathogenic bacteria in juices are sufficiently alarming for immediate action by the competent authorities. To avoid such excessive contamination, street foods, including beverages, must be properly stored and handled in accordance with good hygiene practices (GHP) during sales operations to preserve their quality (4) and avoid harmful effects on the consumer. Regular quality control of fruit juices for consumption on campus should be implemented to reduce the risk of foodborne diseases or other infections harmful to consumers. Many researchers have reported the role of street foods as vectors of pathogenic bacterial transmission to humans. While most studies focus on microbiological and hygienic quality, little effort has been made to identify antibiotic resistance mechanisms and the spread of antibiotic resistance genes, important information for the control of foodborne pathogens (7). Some *E. coli* isolates tested showed resistance to ampicillin, amoxicillin + clavulanic acid, cephalotin, cefotaxime, tetracycline and sulfonamide. These results are similar to those obtained by Somda et al (24) when testing strains of *E. coli* isolated from chicken meat in Ouagadougou, Burkina Faso. *Salmonella spp*. isolates are 100% sensitive to all antibiotics tested. These results are in contrast to those of Bodering et al (40) who reported high resistance to some antibiotics (cefotaxime, amoxicillin+ clavulanic acid, cephalotin) and those of Nemo et al (41) who reported resistance of these strains to ampicillin and tetracycline in Ethiopia. Our *S. aureus* isolates were 100% resistant to ampicillin, aztreonam, cefotaxime and tetracycline. This result is close to that of Nemo et al (41) who reported resistance of *S. aureus* to chloramphenicol at

95.74% and ciprofloxacin at 93.62%. In our study, 21.42% of bacteria isolated from drinks developed resistance to more than three antibiotics from different families and constitute Multi-Resistant Bacteria (MRB). This percentage is lower than that (36%) obtained by Ahoyo et al (14) in Benin who isolated 325 strains of potentially pathogenic bacteria with 36% multi-resistant to antibiotics, including 44% broad spectrum beta-lactamase-producing *Escherichia coli* (ECBLSE), 32% methicillin-resistant *Staphylococcus aureus* and 24% vancomycin-resistant enterococci. BMRs are often isolated in hospitals with frequencies comprised between 12 and 28.7% as reported by Ahoyo et al (14) and are responsible for severe nosocomial infections. Today, we are witnessing their proliferation in the community, especially in foodstuffs, with frequencies of 58% and 88% among bacteria indicating faecal contamination; found respectively by Persoons et al (42) in Belgium, then Megan et al (43) in the United States with a possible transmission of strains of animal origin to humans through food. Our results are of the same order of magnitude as the hospital data and are worrying because they pose a real health problem for the student world and the entire university community. The emergence of BMRs may be the result of the massive and uncontrolled use of antibiotics.

5.Conclusion

In Benin, the increasing rate of consumption of street foods leads people to a right to expect that the food they consume is safe and suitable for consumption. The food sold on the campuses of the University of Abomey-Calavi is the most accessible source of food for students, teachers, civil servants, etc. They are cheap, varied and available everywhere. However, they have the disadvantage of presenting risks of food poisoning due to their microbial contamination. Our study, which aims to evaluate the microbiological quality of mass consumption drinks sold in mass catering on the campuses of the University of Abomey-Calavi, revealed a high non-compliance rate for the bacteriological quality of the drinks analysed. This high rate may be due to non-compliance with good hygiene practices during the manufacture and production of beverages. In addition, the hygiene status of food handlers can also contributes to the poor microbiological quality and safety of these products. Lack of knowledge, insufficient or incorrect application of good hygiene and manufacturing practices by sellers at the man power, environment, working material, raw material and methodology levels leads to biological, chemical and physical contamination of beverages. These results show that a significant effort has been made to improve the hygienic quality of food sold on campuses. However, there should be continuity and sustainability in the application of hygiene rules by all the actors involved (sellers, consumers). Food sold on the street can be a source of foodborne pathogen microorganims. Practical measures through the education and awareness of sellers about good hygiene practices during the production and sale of food must be highlighted in order to avoid foodborne diseases.

Conflict of interest

Authors declare that there is no conflict of interest.

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References:

1.Kouassi KA, Dadi AT, N'guessan K, et al. Conditions hygiéniques des vendeurs et affections liées à la consommation de la viande bovine cuite vendue aux abords des rues de la ville d'Abidjan (Côte d'Ivoire). Microbiol Hyg Alim 2012; 24:15-20.

2.Barro N, Ouattara CA, Nikiema PA, et al. Evaluation de la qualité microbiologique de quelques aliments de rue dans la ville de Ouagadougou au Burkina Faso. Cahiers Etudes Recherches Francophones 2003; 12:369-74.

3.Hiamey SE, Hiamey GA. Street food consumption in a Ghanaian Metropolis: The concerns determining consumption and non-consumption. Food Control 2018; 92:121-127.

4.Massieke AARS, Tapsoba FWB, Kabore D, et al. Etude sur la capacité de production, du circuit de commercialisation et de la consommation du zoom-koom vendu dans la ville de Ouagadougou au Burkina Faso. Int J Biol Chem Sci 2017; 11:2294-2305.

5.Acho-Chi C. The mobile street food service practice in the urban economy of kumba, Cameroon. Singapore J Trop Geogr 2002; 23:131-148.

6.FAO, 2016. Street Food Vending in Accra, Ghana. Field survey report. Editors Marras S, Bendech AM and Laar A, Editors. University of Ghana, 57p.

7.Lin L, Wang SF, Yang TY, et al. Antimicrobial resistance and genetic diversity in ceftazidime non-susceptible bacterial pathogens from ready-to-eat street foods in three Taiwanese cities. Sci Rep 2017; 7:15515.

8.Tabassum A, Saha ML, Islam MN. Prevalence of multi-drug resistant bacteria in selected street food and water samples. Bangladesh J Bot 2018; 44:621-627.

9.Bezzar N. Caractérisation génétique par réaction de polymérisation en chaîne (PCR) des souches de Staphylococcus aureus d'origine hospitalière. Université de la Republique Algérienne Démocratique Populaire. Available at: http://dspace.univ-tlemcen.dz/112/6442/1/ bitstream/BEZZAR-Nesrine.pdf Cited : 2014.

10.Athira CK, Milton AAP, Reddy A, et al. Diversity of toxin-genotypes among Clostridium Perfringens isolated from healthy and diarrheic neonatal cattle and buffalo calves. Anaerobe 2018; 49: 99-102.
11.Kashosi TM, Muhandule AB, Mwenebitu DL, et al. Antibio-résistance des souches de Salmonella spp isolées d'hémocultures à Bukawu en RD Congo. Pan Afr Med J 2018; 29: 42.
12.Garas LC, Cooper CA, Dawson MW, et al. Young pigs consuming lysozyme transgenic goat milk are protected from clinical symptoms of enterotoxigenic *Escherichia coli* infection. J Nutr 2017; 141: 2050-2059.
13.WHO. antimicrobial Resistance. Available at https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance. Cited: October 29, 2018.
14.Ahoyo TA, Ahissou H, Kounon F, et al. Etude de la qualité bactériologique des aliments vendus sur le campus de l'Université d'Abomey-Calavi au Bénin. Int J Biol Chem Sci 2010; 4: 1083-1092.
15.SHSA : Service Hygiène et Sureté des Aliments, 2017. Rapport du Service Hygiène et Sureté des Aliments : Etat des lieux des installations de vente des denrées alimentaires, et d'autres prestations de service sur les campus de l'Université d'Abomey-Calavi. University of Abomey-Calavi, 60p.
16.NF EN ISO 6579-1, 2017. Microbiologie des aliments – Méthode horizontale pour la recherche des Salmonella spp. (Indice de classement: V08-013).
17.NF EN ISO 6888-3, 2003. Microbiologie des aliments- Méthode horizontale pour le dénombrement des Staphylocoques à coagulase positive (Staphylococcus aureus et autres espèces) - Partie 3 : Recherche et dénombrement par la méthode NPP pour les faibles nombres.
18.NF ISO 4833-2, 2013. Microbiologie des aliments— Méthode horizontale pour le dénombrement des micro-organismes –Partie2 : Comptage des colonies à 30°C.
19.NF ISO 16649-2, 2001. Microbiologie des aliments – Méthode horizontale pour le dénombrement des Escherichia coli B-glucuronidase positive – Partie 2 : technique de comptage des colonies à 44°C au moyen de 5-bromo-4-chloro-3-indolyl B-D-glucuronate (Indice de classement: V08-031-2).
20.NF ISO 21527-1, 2008. Microbiologie des aliments – Méthode horizontale pour le dénombrement des levures et moisissures – Partie 1 : technique par comptage des colonies dans les produits à activité d'eau supérieure à 0,95 (Indice de classement : V08-040-1).
21.NF ISO 21527-2, 2008. Microbiologie des aliments – Méthode horizontale pour le dénombrement des levures et moisissures – Partie 2 : technique par comptage des colonies dans les produits à activité d'eau inférieure ou égale à 0,95 (Indice de classement : V08-040-2).
22.NF V08-060, 2009. Microbiologie alimentaire- Dénombrement des coliformes thermotolérants par comptage des colonies obtenues à 44°C.
23.NF V08-61, 2009. Microbiologie des aliments- Dénombrement en anaérobiose des bactéries sulfito-réductrices par comptage des colonies à 46°C.
24.Somda NS, Bonkoungou OJ, Zongo C, et al. Safety of ready-to-eat chicken in Burkina Faso: Microbiological quality, antibiotic resistance, and virulence genes in Escherichia coli isolated from chicken samples of Ouagadougou. Food Sci Nutr 2018; 1077-1084.
25.Chong SY, Rao PV, Soon JM. Identification of Escherichia spp. strains in street-vended beverages and associated preparation surfaces using 16S rRNA analysis. Int Food Res J 2017; 24:1811-1818.
26.CLSI: Clinical and Laboratory Standards Institute, 2011. CLSI document M100-S21. Performance standards for antimicrobial susceptibility testing, 20th ed informational supplement, Wayne, Pa.
27.CLSI: Clinical and Laboratory Standards Institute, 2016. CLSI document M100-S21. Performance standards for antimicrobial susceptibility testing, 26th ed informational supplement, Wayne, Pa
28.EUCAST: European Committee on Antimicrobial Susceptibility Testing, 2018. Détermination de la sensibilité aux antibiotiques, résistances naturelles aux antibiotiques des principales espèces, bactériennes d'intérêt médical, définition des catégories cliniques, concentrations critiques pk/pd, non reliées à une espèce, tableaux des concentrations critiques pour l'interprétation des cmi et des diamètres critiques des zones d'inhibition.
29.Gulf Standards, 2000. Microbiological criteria for foodstuffs-Part, GCC, Riyadh, Saudi Arabia.
30.Health Protection Agency, 2009. Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods. London: Health Protection Agency, November 2009.
31.Reddi SL, Kumar RN, Balakrishna N, et al. Microbiological quality of street vended fruit juices in Hyderabad, India and their association between food safety knowledge and practices of fruit juice vendors. Int J Curr Microbiol App Sci 2015; 4:970-982.
32.Tshipamba ME, Lubanza N, Adetunji MC, et al. Molecular Characterization and Antibiotic Resistance of Foodborne Pathogens in Street-Vended Ready-to-Eat Meat Sold in South Africa. J Food Protect 2018; 81:1963-1972.
33.Mahale DP, Khade RG, Vaidya VK. Microbiological analysis of street vended fruit juices from Mumbai city, India. Int J Food Safe 2008; 10:31-34.
34.Mouloudi F, 2013. La qualité microbiologique de la restauration collective : cas de restaurant universitaire

d'Oran. Mémoire de master en microbiologie fondamentale. Université d'Oran Es-senia.
35.Khan MM, Islam TM, Chowdhury HMM, et al. Assessment of microbiological quality of some drinks sold in the streets of Dhaka University Campus in Bangladesh. Int J Food Contam 2015; 2: 4.
36.Sylla KSB, Seydi M. Etude de la qualité hygiénique du poisson utilisé en restauration collective universitaire à Dakar (Sénégal). RASPA 2003; 1:17-23.
37.Lewis JE, Thompson P, Rao BV, et al. Human bacteria in street vended fruit juices: A case study of Visakhapatnam City, India. Int J Food Safe 2006; 8:35-38.
38.Lateef A, Julius K, Oloke KJ, et al. The Microbiological Quality of Ice Used to Cool Drinks and Foods in Ogbomoso Metropolis, Southwest, Nigeria. Int J Food Safe 2006; 8: 39-43.
39.Awuor L, Thompson S, Thompson B, et al. Microbiological quality and handling practices of ice served in selected downtown Toronto food premises. Environ Health Rev 2016; 59:8387.
40.Bodering A, Ndoutamia G, Ngandolo BN, et al. Utilisation des antibiotiques et profil de résistance des souches de Salmonella spp. et Escherichia coli isolées des exploitations avicoles des villes de N'Djaména et Doba au Tchad. Int J Biol Chem Sci 2017; 11:1669-1684.
41.Nemo R, Bacha K, Ketema T. Microbiological quality and safety of some-street-vended foods in Jimma Town, Southwestern Ethiopia. Afr J Microbiol Res 2017; 11:574-585.
42.Persoons D, Dewulf J, Smet A, et al. Prevalence and persistence of antimicrobial resistance in broiler indicator bacteria. Microb Drug Resist 2010; 16: 67-74.
43.Megan EJ, Trent FJ, Reinstein SL, et al. Antimicrobial susceptibility of foodborne pathogens in organic or natural production systems: An overview. Foodborne Pathog Dis 2008; 5:721-730.