



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 murlinae*, *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 | Plate Count Agar | NF EN ISO 4833- |
| Flora at 30°C | (PCA OXOID CM0463) | 2 : 2013 |
| Total coliforms at 30°C | Brilliance <i>E. coli</i> OXOID CM01046 | Modified NF ISO 4831 : 2006 |
| Fecal coliforms at 44°C | Brilliance <i>E. coli</i> 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 MgCl₂ (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' CGGCTACCTTGTTTACTT3' 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 2 nd generation |
| Cefotaxime (CTX) 30 µg | Beta lactamine Cephalosporin 3rd generation |
| Chloramphenicol (CHL) 30 µg | Phenolate |
| 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. |
| | Proximity to the point of sale | 34.55 | 12. |
| | Cleanliness of the establishment | 1.82 | 3.5 |
| | Cleanliness of the establishment, Price | 12.73 | 8.8 |
| Source of supply of raw materials | Unique | 14.55 | 9.3 |
| | Various | 85.45 | 9.3 |
| Protection of food sold | Yes | 67.27 | 12. |
| | No | 32.73b | 12. |

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, uncleaned 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 |
| | Trained | 25.45 | 9.17 |
| 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 gentamycin 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.

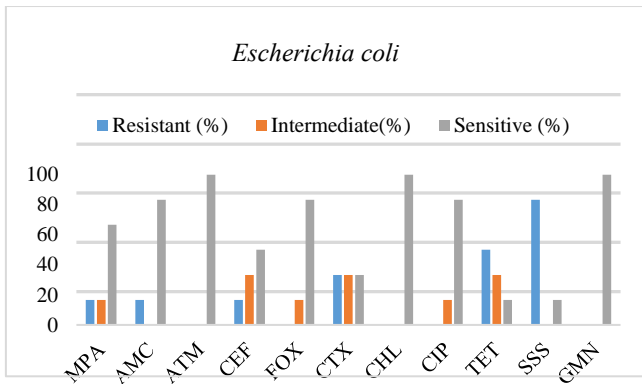


Figure 1: Antibiotic resistance of *E. coli* (n=6) isolated from "Degue" beverages

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

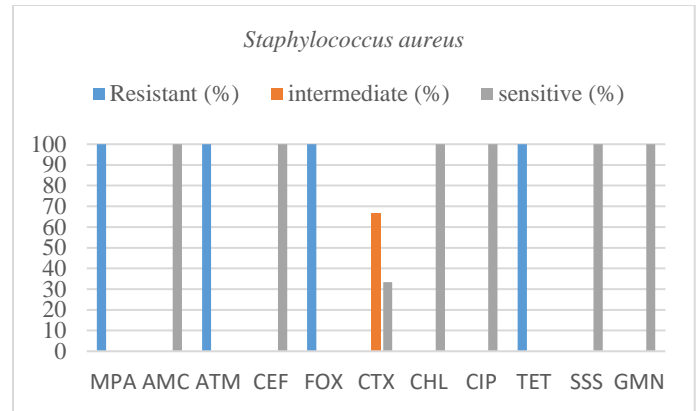


Figure 2: Antibiotic resistance of *Staphylococcus aureus* isolated from beverages (n=3)

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

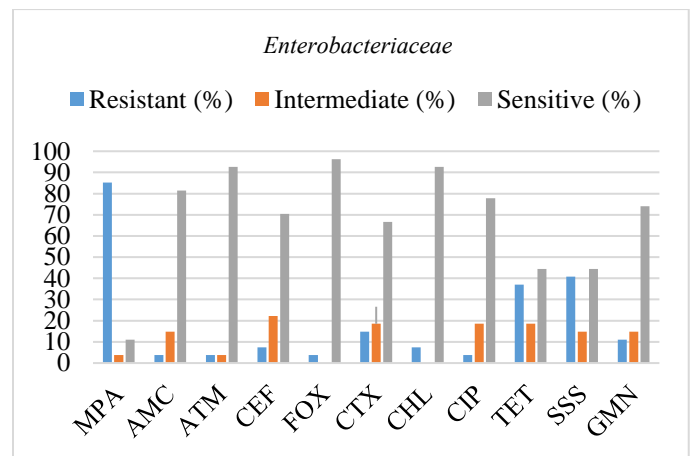


Figure 3: Antibiotic resistance of other *Enterobacteriaceae* isolated from beverages samples (n=27)

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 (log ₁₀ cfu/mL) | Total coliforms (log ₁₀ cfu/mL) | Fecal coliforms (log ₁₀ cfu/mL) | <i>E. coli</i> (log ₁₀ cfu/mL) | Yeast (log ₁₀ cfu/mL) | Moulds (log ₁₀ cfu/mL) | <i>Coagulase positive Staphylococcus</i> (log ₁₀ cfu/mL) | <i>Salmonella</i> spp (Absence in 25ml) | ASR (log ₁₀ 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 2 nd generation | Cephalosporin 3rd generation | | | | | | |
| | AMP | AMC | ATM | CEF | FOX | CTX | GMN | CIP | TET | CHL | SSS |
| <i>E. coli</i> * | R | S | S | S | S | S | S | S | R | S | R |
| <i>Y. intermedia</i> * | R | S | S | R | S | R | R | I | I | S | R |
| <i>K. ascorbata</i> * | R | S | S | S | S | S | S | S | R | S | R |
| <i>K. Georgiana</i> * | R | S | S | I | S | I | S | S | R | S | R |
| <i>C. murlinae</i> * | R | S | S | S | S | S | S | S | R | S | R |
| <i>K.pneumonia</i> ** | R | I | S | S | S | R | S | I | R | R | R |
| <i>K.pneumonia</i> *** | R | I | S | I | S | I | R | S | R | R | R |
| <i>K.pneumonia</i> *** | R | S | S | S | S | S | S | S | R | S | R |
| <i>K.pneumoniae</i> *** | 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 microorganisms 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/cm² 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. sciuri*, 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 Boderling 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 contribute 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 microorganisms. 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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