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Isolation, characterization and antibiotic susceptibility of *Escherichia coli* and *Salmonella* spp. isolated from local beverages (bissap, gnamakoudji) sold in Ouagadougou, Burkina Faso

Gertrude Bsadjo Tchamba[1*], Hadiza Ibrahim Bawa[1], Ariane Nzouankeu[2], Touwendsida Serge Bagré[1], Alfred S. Traoré[1], Nicolas Barro[1]

[1]*Laboratoire de Biologie Moléculaire, d'Epidémiologie et de Surveillance des Bactéries et Virus Transmissibles par les Aliments (LaBESTA), Centre de Recherches en Sciences Biologiques, Alimentaires et Nutritionnelles (CRSBAN), Ecole Doctorale Sciences et Technologies, 03 BP 7021 Ouagadougou 03, Université de Ouagadougou, Burkina Faso*

[2]*Centre Pasteur du Cameroun, BP 1274 Yaoundé, Cameroun*

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Abstract

Bissap» and «gnamakoudjii» are two local beverages highly produced and consumed in Burkina Faso, but potential hazard related to unhygienic contamination can cause a major risk to the health of consumers. This study was performed to assess the prevalence and antibiotic resistance of *Salmonella* spp. and *Escherichia coli* in 150 samples of «gnamakoudjii» and 150 samples of «bissap» sold in twenty four open markets in Ouagadougou city. Samples were aseptically collected and analyzed using standard microbiology methods within an hour of procurement. Slide agglutination with 12 enteropathogenic *E. coli* (EPEC) antisera was used for the detection of EPEC. Nineteen (12.70%) and thirty one (20.70%) strains of *E. coli* were respectively isolated in «bissap» and «gnamakoudjii». Among *E. coli* isolated, 7 (14%) *E. coli* isolates were serotyped as EPEC belonging to 4 serotypes (*E. coli* O86; *E. coli* O119; *E. coli* O126 and *E. coli* O128). No *Salmonella* were detected in «gnamakoudjii» while 2 (1.33%) strains of *Salmonella* were isolated in «bissap». Antibiotic resistance results indicated that *E. coli* isolates were resistant to amoxicillin-clavulanate, chloramphenicol, cefamandole, aztreonam, cephalothin, nalidixic acid, ticarcillin and tetracycline. Training/retraining on hygiene and sanitation is recommended to avoid contamination of local beverages.

* **Corresponding Author:** Gertrude Bsadjo Tchamba ✉ bsadjotchamba@gmail.com

Introduction

Traditional beverages are largely consumed in several countries of Africa and attract all the age groups (Cisse *et al.*, 2009; Osuntogun and Aboaba, 2004; Barro *et al.*, 2002). Calyces of the Roselle plant (*Hibiscus sabdariffa* var *sabdariffa*) and ginger plants (*Gingiber officinalis* Roscoe) are respectively used for the production of two traditional beverages and tonic without alcohol: «bissap» and «gnamakoudjii».

The non-alcoholic nature of these beverages makes them to be well appreciated by consumers as whole (Osuntogun and Aboaba, 2004). Health benefits of these local beverages are many, owing to the various properties present in them. The Roselle plant is said to be antiseptic, diuretic and aphrodisiac (Osuntogun and Aboaba, 2004). The drink made by placing the calyx in water is said to be folk remedy for cancer (Osuntogun and Aboaba, 2004). Ginger and its constituents are reported to have analgesic effect, to have significant Hepatoprotective activity etc (Nwachukwu and Aniedu, 2013; El-Sharaky *et al.*, 2009; Mendi *et al.*, 2009; Nurtjahja-Tjendraputra *et al.*, 2003).

Despite their nutritive, medicinal value, minerals and vitamin content, «bissap» and «gnamakoudjii» could often associated with food-borne pathogens such as *Staphylococcus aureus, Salmonella* spp. and *Escherichia coli* (Osuntogun and Aboaba, 2004; Barro *et al.*, 2002).

The prevalence of antimicrobial resistance among food-borne pathogens has increased during recent decades. This increase is attributed to the empiric use of antibiotics by humans in developing countries (Rashed *et al.*, 2013; Lateef *et al.*, 2006). Absence of training about HACCP prerequisites such as good practices, poor hygienic practices, unsafe local beverages storage temperatures (dressing with ice prolonged preservation without refrigeration), cross-contamination and preparation under unhygienic conditions by the vendors who are by and large illiterate and have poor personal hygiene, increases greatly the risk of local beverage contamination (Bsadjo Tchamba *et al.*, 2014; Nwachukwu and Aniedu, 2013; Tasnim *et al.*, 2010; Barro *et al.*, 2007; Lewis *et al.*, 2006; Osuntogun and Aboaba, 2004; Barro *et al.*, 2002). Water is the main component of local beverages. Unhygienic water for dilution can also be a major source of pathogenic contamination due to unhygienic storage and handling (Tasnim *et al.*, 2010; Lewis *et al.*, 2006). Infections due to *Salmonella* spp. and *E. coli* remain a global problem. These infections may cause significant morbidity and mortality as well as considerable economic losses.

In view of the great demand of «bissap » and «gnamakoudjii », this study was conducted to investigate the microbiological quality of these two traditional beverages sold in open markets of Ouagadougou, Burkina Faso and to assess the antibiotics susceptibility of *E. coli* and *Salmonella* spp. strains isolated from different samples.

Material and methods

Samples collection and their processing

During the study, twenty four (24) open markets in five (5) municipalities (Baskuy, Bogodogo, Boulmiougou, Nongre-Massom and Sig-Noghin) of Ouagadougou city were chosen for samples collection. Three hundred (300) local beverages samples, 150 «bissap » and 150 «gnamakoudjii », of approximately 300 ml were randomly collected in several point of sale of different open markets from October 2011 to December 2012 (Table 1). All samples were collected regularly in sterile containers, kept in ice box, maintained at 4°C during transportation and were analyzed within two hour of collection.

Microbiological analysis

The ISO 6579:2002(E) and ISO 4832:1991 (F) methods lightly modified were used respectively for isolation and identification of Salmonella spp. and Escherichia coli.

Enrichment broths

Briefly, twenty-five millilitres (25 ml) of «bissap » or «gnamakoudji» samples were combined with 225 ml

of buffered peptone water (Liofilchem, Italy), mixed for 2 min with vortex (HEIDOLPH, Germany) and incubated at 37°C for 24 hours. For detection of *Salmonella* spp. the preenrichment broth culture (0.1ml) was inoculated into 10 ml of rappaport vassiliadis soy broth (Liofilchem, Italy) which was incubated at 42°C for 24 hours.

Bacteria isolation procedures

For *Salmonella* spp. two loopfuls of each enrichment broth were streaked onto Xylose Lysine Désoxycholate (XLD) agar plates (Liofilchem, Italy) and incubated at 37°C for 18-24 hours. Three to five suspected colonies red with or without black center were selected and tested biochemically by using urease, indole test, orthonitrophenyl-β-D-galactopyranoside (ONPG), citrate, mannitol, mobility, H_2S and fermentative gas production. Finally the selected isolates were suspended in physiological saline solution (NaCl, 9 g/L in water) for confirmation by API 20E system (bioMérieux, France) and interpretation was done according to API20^E catalogue.

For *E. coli* detection, two loopfuls of the pre-enriched broth were streaked onto Eosin Methylene Blue (EMB) agar (Liofilchem, Italy) and incubated at 44°C for 37 hours. Three to five presumptive *E. coli* colonies based on colonies with a metallic-green color and a dark purple center were selected and purified to obtain pure cultures onto Mueller Hinton agar (Liofilchem, Italy) that were subjected to routine primary and biochemical (IMViC tests namely Indole test, Methyl Red test, Voges-Proskauer test and Citrate test). Species identification was performed by determination of the biochemical reaction profile (bioMérieux, France).

Enteropathogenic *Escherichia coli* (EPEC) serotyping

Enteropathogenic *E. coli*, were further confirmed by slide agglutination test using nonavalent, trivalent and monovalent antisera (BIORAD, France). Detection of the O- antigen is performed by slide agglutination according to the instructions of the manufacturer. *E. coli* isolates were tested with twelve different antisera for the detection of EPEC. Antibodies in the specific sera agglutinate with the bacteria when the corresponding antigens are present. Only strong agglutination occurring within 1 min was considered to be positive reaction.

Antimicrobial susceptibility testing

Antibiotic susceptibility testing of *Salmonella* spp. and *E. coli* was performed using the Kirby Bauer disc diffusion method (Bauer *et al.*, 1966). Briefly, a single colony of each isolate was introduced into 2 ml of the physiological solution, incubated for 4 hours and the culture turbidity was then adjusted to 0.5 McFarland standard. Sterile cotton swabs were dipped into suspensions and were spread evenly over the entire agar surface. Antibiotics impregnated discs (Liofilchem, Italy): Amoxicillin-clavulanate (AMC 30), ticarcillin (TIC 75), imipenem (IMI 10), aztreonam (ATM 30), cephalothin (KF 30), cefalexin (CL 30), cefamandole (MA 30), ceftriaxone (CRO 30), cefepime (FEP 30), gentamicin (GEN 10), chloramphenicol (C 30), tetracycline (TE 30), nalidixic acid (NA 30) and ciprofoxacin (CIP 5) were then placed onto the surface of the inoculated plates. After incubation at 37°C during 24 hours, diameters of inhibition zones were measured and interpreted as susceptible and resistant using disc diffusion method according to EUCAST (European Committee on Antimicrobial Susceptibility Testing) criteria (EUCAST, 2012). *E. coli* ATCC 25922 was used as a reference strain.

Results and discussion

Contamination of beverages

Bissap» and «gnamakoudjii» sold in Ouagadougou at open markets are contaminated by *E. coli* and *Salmonella* spp. The microbiological results shown that 12.70% of «Bissap» samples and 20.70% of «gnamakoudjii» samples were contaminated by *E. coli* (Table 2). All the «gnamakoudjii » samples investigated were devoid of *Salmonella* spp, whereas this pathogen was isolated from only 2 strains 1.33% of the «bissap» samples tested.

The presence of *Salmonella* spp. could be a matter of

serious concern. The fact that «bissap» processed with boiled water harboured *Salmonella* spp. implies that they were contaminated after production indicating inappropriate manufacturing processes. Indeed, Poojara and Krishna (2012) shown that the presence of this pathogen indicates poor handling and a potential for beverages to cause food borne diseases. The prevalence of E. coli in the «gnamakoudjii» samples compared with «bissap» samples could be explained by the absence of heat treatment in its production process. Although «bissap» undergoes a heat treatment, the practice of addition of some quantity of water to these beverages after step infusion may also be a source of introducing faecal contaminants which may have come from the water itself. This contamination could also result from post process recontamination due to cross-contamination by dirty equipment, unhygienic environment or inappropriate handling. Based on the above studies, it has been showed that water used in the preparation of fruit juices was highly contaminated with faecal coliforms (Bsadjo Tchamba *et al.*, 2014). In addition to this, the contamination of beverages was also due to poor personal and domestic hygiene indicating lack of knowledge of hygienic practices and safety of final products (Rashed *et al.*, 2013; Uma reddy *et al.*, 2009; Mahale *et al.*, 2008; Lewis *et al.*, 2006; Barro *et al.*, 2002).

TABLE 1. Origins and number of samples collected in ouagadougou's markets.

Municipalities	Open markets	Number of samples	
		"Bissap"	"Gnamakoudjii"
Baskuy	« Baskuy »	6	10
	« Paspanga »	3	3
	« Laarlé »	5	9
	« Gounghin »	8	7
	« Sankara-yaare »	4	4
	« Hamdalaye »	14	10
	« Grand Marché »	11	5
	« Samandin »	3	7
	« Oscar yaare »	3	0
Bogodogo	« Dassasgho »	17	15
	« Zogona »	3	2
	« Wemtenga »	8	10
	« Katre yaare »	5	5
	« Patte d'Oie »	2	8
	« 14yaare »	4	6
	« Naby yaare »	6	5
Boulmiougou	« Cissin »	8	5
	« Nonsin laafi yaare »	6	5
	« Pissy »	5	6
NongreMassom	« Somgandé »	7	4
	"Tanghin »	12	5
	« Wayalghin »	2	6
Sig-Noghin	« Kilwin »	3	7
	« Tampouy »	5	6
Total	24	150	150

According to Osuntogun and Aboaba, (2004) it is known that beverages are occasionally temperature abused during distribution, warehousing and marketing. In Ouagadougou, it was observed that safe storage temperature of local beverages at sale point were difficult to maintain. Generally, local beverages sit for too long at the wrong temperature. Ignorance or no respect of cold chain could constitute a major factor in the contamination of beverages which could pose serious health risks to consumers. Indeed, in India and in Burkina Faso, Lewis *et al.* (2006) and Barro *et al.* (2007) reported that poor quality fruit juice and traditional beverages is probably associated with the higher temperatures (30 to 37°C) which

encourage bacteria multiplication.

The vicinity of the sales points with heaps of garbage and sewage attract house-flies when disposable polythene bags, recycled bottles are hand filled with beverages. The influx of house-flies is explained by the sugar found in traditional beverages. Flies may harbour foodborne pathogens such *E. coli* which further increases recontamination of beverages and could constitute a danger to human health. Indeed, the linkages between houseflies and diarrheal diseases have been documented in previous study in Burkina Faso (Barro *et al.*, 2006).

Table 2. Prevalence of *E. coli* and *Salmonella* spp. in «Bissap» and «Gnamakoudjii».

Traditional beverages	Number of samples	Number (%) of positive samples	
		E. coli	Salmonella spp.
«Bissap»	150	19 (12.70%)	02 (1.33%)
«Gnamakoudjii»	150	31 (20.70%)	00 (0.0%)
Total	300	50 (16.66%)	02 (0.66%)

Enteropathogenic *Escherichia coli* (EPEC) serotyping Among the 12 different serotypes of EPEC, 4 were identified. The EPEC strains were shown to belong to the 4 following serotypes (O86; O119; O126 and O128). The most common were found in O119 (6.41%) and O128 (6.41%). One strain (3.22%) of EPEC isolated in «gnamakoudjii» samples belonged to each of the O86 and O126 serotypes. Only one strain (5.26%) belonged to O86 was isolated in «bissap» samples (Table 3).

Table 3. O-serogroups of Enteropathogenic *E. coli* (EPEC) isolates in «bissap» and «gnamakoudjii».

Products	Number of *E. coli* isolates	Serotypes of EPEC isolates Number (%) of serotypes			
		O 86	O 119	O126	O128
«Bissap»	19	1 (5.26%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
«Gnamakoudjii»	31	1 (3.22%)	2 (6.41%)	1 (3.22%)	2 (6.41%)
Total	50	2 (4.0%)	2 (4.0%)	1 (2.0%)	2 (4.0%)

E. coli form part of human intestinal flora, but some strains, such as EPEC can cause severe foodborne disease. According to a study carried out in Malaysia, its presence in food can serve as an indicator of fecal contamination (Norazah *et al.,* 1998). In Burkina Faso, Bonkoungou *et al.* (2011) showed that, the most frequently detected diarrhoeagenic *E. coli* associated with diarrhoea was EPEC. In Burkina Faso with a hot climate, there is an increasing demand for «bissap» and «gnamakoudjii» during family ceremonies, religious events and fasting month of fasting. Sweet enough to be enjoyed by kids, «bissap» also constitute a curiosity for tourists. The presence of EPEC in «bissap» and «gnamakoudjii» which are widely distributed and largely consumed in Burkina Faso could be a potential source of food borne outbreak.

Susceptibility of the isolates to antibiotics

The resistances of the bacterial isolates to different antibiotics are summarized in Table 4. Susceptibility testing showed that all *Salmonella* spp. isolates were not resistant to all antibiotics tested except tetracycline. *E. coli* isolated from «bissap» and «gnamakoudjii» samples were both not resistant to any of the following antibiotics (cefalexin, imipenem, gentamicin and ciprofloxacin) tested. None of the *E. coli* isolates from «bissap» samples was resistant to ceftriaxone. Resistance to cephalothin and cefepime was not detected in the *E. coli* isolates from «gnamakoudjii» samples. The antibiotics such as aztreonam, cefamandole and chloramphenicol have a resistance of less than 25%. *E. coli* strains isolated from «bissap» and «gnamakoudjii» in this study were highly resistant to tetracycline (78.90% and 45.20%) and ticarcillin (36.80% and 45.20%).

Table 4. Resistances of *E. coli* and *Salmonella* spp. isolated.

Antimicrobials	*Escherichia coli*		*Salmonella* spp.
	B (n=19)	G (n=31)	B (n=02)
	Number (%) of resistance strains		
Amoxicillin-clavulanate	1 (5.30%)	8 (25.80%)	-
Ticarcillin	7 (36.80%)	14 (45.20%)	-
Cephalothin	4 (21.10%)	-	-
Cefalexin	-	-	-
Cefamandole	2 (10.50%)	1 (3.20%)	-
Ceftriazone	-	1 (3.20%)	-
Cefepime	1 (5.30%)	-	-
Imipenem	-	-	-
Aztreonam	3 (15.80%)	5 (16.10%)	-
Gentamicin	-	-	-
Chloramphenicol	1 (5.30%)	4 (12.90%)	-
Tetracycline	15 (78.90%)	14 (45.20%)	2 (100%)
Nalidixic-acid	5 (26.30%)	3 (9.70%)	-
Ciprofloxacin	-	-	-

Legend: B: «Bissap», G: « Gnamakoudjii», -: None.

Concerning *E. coli* O86 strain isolate in «bissap» sample, resistance has been found for the following antimicrobial agents: tetracycline, ticarcillin, aztreonam and amoxicillin-clavulanate. Regarding EPEC isolates in «gnamakoudjii» samples, strain of *E. coli* O86 isolates was found to be resistant to tetracycline, ticarcillin, amoxicillin–clavulanate and to aztreonam. Strains of *E. coli* O119 revealed that 50% (1/2) was resistant to aztreonam while 100% (2/2) were resistant to tetracycline and ticarcillin of the antimicrobials tested. The susceptibility of *E. coli* O128 when tested against tetracycline, ticarcillin and nalidixic acid was 50% (1/2) while aztreonam exhibited 100% (2/2) of resistance. The *E. coli* strain O126 was found to be resistant to aztreonam only.

Antibiogram of isolates revealed varying levels of resistance to the antibiotics tested. The prevalence of resistant strains of *E. coli*, in «bissap» and «gnamakoudjii» is a reflection of the use and misuse of the antibiotics in the society. *Escherichia coli* is an important gastrointestinal flora which has been known to be capable of accepting and transferring plasmids and these plasmids can be transferred readily under stress to other species. Therefore, this attribute has made *E. coli* to be considered as an important reservoir of transferable antibiotic resistance (Aibinu *et al.,* 2007). The selection and spread of resistant organisms in developing countries, which can often be traced to complex socioeconomic and behavioural antecedents, has contributed to the escalating problem of antibiotic resistance worldwide (Aibinu *et al.,* 2007). Increase in antibiotic resistance level is now a global problem. Antimicrobial resistant strains of *Salmonella* spp. and *E. coli* isolated in this study can colonize the customer by consumption of contaminated «bissap» and «gnamakoudjii» and could cause extremely serious public health problems.

Conclusion

Dangerous and resistant bacteria have been isolate in highly consumed beverages. It is necessary to pay more attention to food hygiene practices by avoiding poor handling and raising the awareness of food handlers to good hygiene practices. It is imperative that actions should be taken in order to reduce or eliminate the risk of pathogenic bacteria infection originating from local beverages.

Conflict of Interests

The authors have not declared any conflict of interests.

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