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Occurrence and antimicrobial susceptibility of *Escherichia coli* and *Salmonella* spp. isolated from "zoom-koom" beverage and ice in Ouagadougou, Burkina Faso

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Pathogenic bacteria contaminate beverages and make consumers to have diarrhoeal diseases. This study was carried out to assess the occurrence and antimicrobial susceptibility of *Escherichia coli* and *Salmonella* spp. isolated from "zoom-koom" beverages and ice from open markets of Ouagadougou. One hundred samples of both "zoom-koom" and ice were analyzed using standard microbiological methods. Identification of serotypes of *E. coli* was completed using specific antisera. Strains were subjected to antimicrobial susceptibility test using agar diffusion method. *E. coli* was isolated in 36% "zoom-koom" and 35% ice samples. Among the 71 *E. coli* strains of both samples, there was 16.8% enteropathogenic *E. coli* (EPEC), belonging to six serotypes: *E. coli* O26, *E. coli* O55, *E. coli* O86, *E. coli* O119, *E. coli* O126 and *E. coli* O128. *Salmonella* was also found in 1% "zoom-koom" and 2% ice samples. Antibiotic susceptibility revealed that *E. coli* isolates resist amoxicillin-clavulanate, ticarcillin, cephalothin, cefamandole, ceftriaxone, cefepime, aztreonam, gentamicin, chloramphenicol, tetracycline, nalidixic acid and ciprofloxacin at different time. Meanwhile, *Salmonella* strains were sensitive to all tested antibiotics except tetracycline. The presence of EPEC and *Salmonella* spp. in the samples indicates that hygiene practices are being improved during processing to reduce the risk of having infection when they are consumed.

Keys words: "Zoom-koom"; ice, *Escherichia coli*, enteropathogenic *E. coli* (EPEC), *Salmonella*, antibiotics, Burkina Faso.

INTRODUCTION

Traditional beverages linked to the ancestral culinary practices in Africa have recently become popular in street trading in many developing countries such as Burkina Faso. "Zoom-koom" is a traditional sweetened beverage produced

from millet (*Penissetum glaucum*) flour. "Zoom-koom" produced essentially by handling and an unstandardized operation is one of common street-vended beverage. The millet grains are washed and dried. Some quantities of black pepper are added to them, after which they are ground into flour. The millet flour is then mixed with water. The resulting mixture is passed through a wet mesh sieve or a clean muslin cloth; this may sometimes be done in unhygienic environmental conditions, making it susceptible to contamination from flies. After filtration, ginger, lemon juice or pineapple and sugar are then added to the filtrate to give it taste, according to the will of manufacturer. 'Zoom-koom' is chilled in refrigerator or by using edible ice; then it becomes ready for consumption. It is usually sold using hand-filling glass cups and sometimes with hand tied in some disposable polythene bags or packaged in 0.5 to 1.5 L recycled plastic bottles. Many vendors reuse water to rinse the glass cups after each use. Poor handling practices, cross contamination during preparation and sales and ambient storage temperature could contribute to the presence of microbial pathogens in traditional beverages. The use of low quality water for the preparation of traditional beverages, reusing water to wash glass cups at the sales point, selling beverages without adequate protection and without good hygienic practices lead to pathogenic contamination (Barro et al., 2002; Lewis et al., 2006; Elmahmood and Doughari, 2007; Titarmare et al., 2009; Rashed et al., 2013). "Zoom-koom" is not only largely consumed for its characteristic aroma and nutritious value, but also for its non-alcoholic nature. Indeed, Compaore et al. (2011), in a previous study in Burkina Faso, showed that millet flour is rich in calcium, carbohydrates and proteins. It is also used in cultural and religious ceremonies such as baptisms and funerals in West African countries, especially in Burkina Faso. This beverage is often served with ice liberally to the thirsty customers.

Water is the most important raw material in the ice production business. Ice is often packaged and sold in transparent polythene nylon bags. Since ice used to refrigerate soft drinks is directly added to the beverages, it also needs to be safe for consumption (Falçao et al., 2002). When ice is thawed the microorganisms remaining may be injured, but they tend to recover their viability so that when the ice melts into drinks, they may be able to survive there too (Lateef et al., 2006). Many studies showed the association between contaminated ice, consumption of traditional beverages and food borne diseases due to pathogenic microorganisms such as: *Vibrio cholerae*, *Shigella* spp., *Salmonella* spp. and *Escherichia coli* (Agbessi et al., 2001; Falçao et al., 2002; Lateef et al., 2006; Mahale et al., 2008; Sunday et al., 2011).

It is now generally accepted that the main risk factor for the increase in resistance in pathogenic bacteria is the increased use of antibiotics. Use of antimicrobial agents in any environment creates selective pressures that favour the survival of antibiotic resistant pathogens (Nipa et al., 2011). The prevalence of antimicrobial resistance among food and beverages pathogens has increased in recent decades and become a major threat to public health. Previous studies have shown that ice and beverages can be contaminated with pathogenic bacteria (Lateef et al., 2004; Lateef et al., 2006; Rashed et al., 2013). Due to the substantial increase in resistance to antibiotics, it is essential to monitor the antibiotic susceptibility of pathogens in various food sold on the streets, especially traditional beverages and ice.

This study was designed to evaluate the prevalence and antimicrobial resistance of *Escherichia coli* and *Salmonella* serotypes in sample of "zoom-koom" and edible ice sold in Ouagadougou, the capital city of Burkina Faso.

MATERIALS AND METHODS

Study area and sampling collection

The study was conducted in the 18 major open markets in the five municipalities of Ouagadougou, from October 2011 to December 2012. In each of these markets, there are several sales points. One hundred (100) "zoom-koom" samples and one hundred (100) edible ice samples of approximately 300 ml were collected regularly in sterile containers; they were kept at 4°C and analyzed within 2 h after sampling. Each "zoom-koom "and ice samples was collected at the same points and time (Table 1).

Sampling processing

Isolation and identification of *Salmonella* and *Escherichia coli*

The ISO 6579:2002(E) and ISO 4832:1991 (F) methods lightly modified were used respectively for isolation and identification of *Salmonella* spp. and *E. coli*. Twenty-five milliliters (25 ml) of "zoom-koom" or edible ice samples were homogenized with 225 ml of sterile buffered peptone water (Liofilchem, Italy), and incubated at 37°C for 24 h. For *Salmonella* isolation, 0.1 ml of pre-enriched broth culture was homogenised in 10 ml of rappaport vassiliadis soy broth (Liofilchem, Italy) and incubated for 24 h at 42°C.

For *E. coli* isolation, two loopfuls of pre-enriched broth was streaked onto Eosin Methylene Blue (EMB) agar (Liofilchem, Italy) and incubated at 44°C for 24 h. Three to five presumptive metallic-green colonies were selected, purified by streaking onto Mueller Hinton agar (Liofilchem, Italy) and then tested by IMViC tests (Indole test, Methyl Red test, Voges Proskauer test and Citrate) and API 20E (bioMérieux, France).

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Table 1. Samples of "Zoom-koom" and ice in Ouagadougou markets.

Municipalities	Markets	Number of samples	
		"*Zoom-koom*"	Edible ice
Baskuy	"Oscar yaar"	5	5
	"Grand marché"	5	5
	"Samandin"	5	5
	"Laarlé"	8	8
	"Sankara yaar"	7	7
	"Hamdalaye"	10	10
	"Baskuy yaar"	6	6
Bogodogo	"Naby yaar"	5	5
	"Wemtenga"	6	6
	"14 yaar"	5	5
Boulmiougou	"Cissin"	5	5
	"Nonsin laafi yaar"	5	5
	"Pissy"	5	5
NongreMassom	"Somgande"	4	4
	"Tanghin"	3	3
	"Wayalghin"	6	6
Sig-Noghin	"Kilwin"	5	5
	"Tampouy"	5	5
Total	18	100	100

For *Salmonella* spp. isolation, two loopfuls of enriched broth were streaked onto Xylose Lysine Deoxycholate (XLD) agar (Liofilchem, Italy) and incubated at 37°C for 18-24 h. Three to five suspected colonies (Red colonies with or without black centre) were selected and tested biochemically by using urease, indole test (BIO-RAD, France), orthonitrophenyl-β-D- galatopyranoside (ONPG), citrate, mannitol, mobility, hydrogen sulphur (H_2S) and fermentative gas production. Finally, the selected isolates were suspended in physiological saline solution (NaCl, 9 g/L) for confirmation by API 20E system (bioMérieux, France) and interpretation was done according to API 20E catalogue.

EPEC Serotyping

Enteropathogenic *E. coli* was confirmed by slide agglutination test using nonavalent, trivalent and monovalent antisera according to the instructions of the manufacturer (BIO-RAD, France). Twelve different serotypes were tested: trivalent I (O111 + O55 + O26), trivalent II (086 + O119 + O127), trivalent III (O125 + O126 O128) and trivalent IV (O114 + O124 + O142). O- antigen is detected by slide agglutination 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

Antimicrobial susceptibility testing was performed for all isolates using the disc diffusion method as described by Bauer et al. (1966). The pure inoculum of *E. coli* or *Salmonella* spp. strains were prepared by suspending colonies into the physiological solution from agar plates and every suspension was adjusted to 0.5 McFarland standard. Diameters of inhibition zones were determined according to the European committee on Antimicrobial Susceptibility instructions (EUCAST, 2012).

The following antibiotic discs (Liofilchem, Italy) were classified according to ten classes: Aminopenicillins (amoxicillin-clavulanate, 30 µg); Carboxypenicillins (ticarcillin, 75 µg); Carbapenemes (imipenem, 10 µg); Monobactam (aztreonam, 30 µg); Cephalosporins (cephalothin, 30 µg), cefalexin (30 µg), cefamandole (30 µg), ceftriaxone (30 µg), cefepime (30 µg)); Aminoglycosides (gentamicin, 10 µg); Phenicoles (chloramphenicol, 30 µg); Cyclines (tetracycline, 30 µg); Quinolones (nalidixic acid, 30 µg); and Fluoroquinolones (ciprofloxacin, 5 µg). After incubation, the inhibition zones were measured and tested isolates were categorized as sensitive or resistant according to the EUCAST criteria. The inhibition zones of *E. coli* were controlled with the reference sensitivity of *Escherichia coli* ATCC 25922.

RESULTS

Occurrence of *E. coli* and *Salmonella* spp.

The results revealed that out of 100 "zoom-koom" samples examined, *Salmonella* spp. was present in 1 (1%) sample, while *E. coli* was present in 36 (36%) samples. It also showed that *Salmonella* spp. was found in 2 (2%) of the 100 samples of edible ice while *E. coli* was present in 35 (35%) samples (Table 2).

Table 2. Prevalence of *E. coli* and *Salmonella* spp. in "zoom-koom" and edible ice.

Traditional drinks	Number of samples	Number (%) of positive samples	
		E. coli	*Salmonella* spp.
"Zoom-koom"	100	36 (36.0%)	01 (1.0%)
Edible ice	100	35 (35.0%)	02 (2.0%)
Total	200	71 (35.5%)	03 (1.5%)

Table 3. O-serogroups of Enteropathogenic *E. coli* (EPEC) isolates in "zoom-koom" and edible ice samples.

Products	Number of *E. coli* isolates	Serotypes of EPEC isolates Number (%) of serotypes					
		O26	O55	O86	O119	O126	O128
"Zoom-koom"	36	0(0%)	0(0%)	2(5.5%)	1(2.7%)	2(5.5%)	1(2.7%)
Edible ice	35	1(2.8%)	1(2.8%)	2(5.7%)	1(2.8%)	0(0%)	1(2.8%)
Total	71	1(1.4%)	1(1.4%)	4(5.6%)	2(2.8%)	2(2.8%)	2(2.8%)

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

Antimicrobials	*Escherichia coli*		*Salmonella* spp.	
	ZK (n=36)	EI (n=35)	ZK (n=1)	EI (n=2)
	Number (%) of Resistance strains			
Amoxicillin- clavulanate	4 (11.10%)	7 (20.00%)	-	-
Ticarcillin	23 (63.90%)	20 (57.10%)	-	-
Cephalothin	1 (2.80%)	1 (2.90%)	-	-
Cefalexin	-	-	-	-
Cefamandole	2 (5.60%)	3 (8.60%)	-	-
Ceftriaxone	2 (5.60%)	4 (11.40%)	-	-
Cefepime	1 (2.80%)	2 (5.70%)	-	-
Imipenem	-	-	-	-
Aztreonam	8 (22.20%)	10 (28.60%)	-	-
Gentamicin	1 (2.80%)	2 (5.70%)	-	-
Chloramphenicol	3 (8.30%)	3 (8.60%)	-	-
Tetracycline	23 (63.90%)	21 (60.00%)	1 (100%)	2 (100%)
Nalidixic-acid	5 (13.90%)	5 (14.30%)	-	-
Ciprofloxacin	1 (2.80%)	1 (2.90%)	-	-

ZK, "Zoom-Koom"; EI, Edible Ice; -, None.

EPEC serotyping

Of the 71 (35%) isolates of *E. coli* in "zoom-koom" beverage and ice, EPEC was identified in 12 (16.8%) samples. Six (6) different serotypes of EPEC were identified: *E. coli* O86 (5.6%), followed by *E. coli* O119 (2.8%); *E. coli* O126 (2.8%) and *E. coli* O128 (2.8%). The less prevalent serotypes were *E. coli* O26 (1.4 %) and *E. coli* O55 (1.4 %). *E. coli* O26 (2.8 %) and *E. coli* O55 (2.8%) serotypes were only detected in edible ice while *E. coli* O126 (5.5 %) was detected in "zoom-koom" (Table 3).

Antibiotics susceptibility

The antibiotic resistance of the bacteria is shown in Table 4. It is noticeable that all *Salmonella* strains were sensitive to all tested antibiotics except two strains which were resistant to tetracycline. Antimicrobial susceptibility test revealed that all *E. coli* strains isolated showed no resistance to cefalexin and imipenem (100%). *E. coli* isolated from "zoom-koom" and ice was resistant to tetracycline (63.90 and 60.00% respectively) and ticarcillin (63.90 and 57.10%); it was moderately resistant to aztreonam (22.20

Table 5. Resistances of enteropathogenic *E. coli* serotyped isolated.

Antimicrobials	Serotypes of EPEC isolates											
	E. coli O26		*E. coli* 055		*E. coli* 086		*E. coli* 0119		*E. coli* 0126		*E. coli* 0128	
	ZK (n=0)	EI (n=1)	ZK (n=0)	EI (n=1)	ZK (n=2)	EI (n=2)	ZK (n=1)	EI (n=1)	ZK (n=2)	EI (n=0)	ZK (n=1)	EI (n=1)
	Number (%) of Resistance strains											
Amoxicillin-clavulanate	None	-	None	-	R(50%)	R(50%)	-	-	-	None	-	-
Ticarcillin	None	-	None	R(100%)	R(50%)	R(50%)	R(100%)	R(100%)	R(50%)	None	R(100%)	R(100%)
Cephalothin	None	-	None	-	-	-	-	-	-	None	-	-
Cefalexin	None	-	None	-	-	-	-	-	-	None	-	-
Cefamandole	None	-	None	-	-	-	-	-	-	None	-	-
Ceftriaxone	None	-	None	-	-	-	-	-	-	None	-	-
Cefepime	None	-	None	-	-	-	-	-	-	None	-	-
Imipenem	None	-	None		-	-	-	-	-	None	-	-
Aztreonam	None	-	None	R(100%)	R(50%)	R(50%)	R(100%)	R(100%)	-	None	R(100%)	R(100%)
Gentamicin	None	-	None	-	R(50%)	R(50%)	-	-	-	None	R(100%)	R(100%)
Chloramphenicol	None	-	None	-	-	-	-	-	-	None	-	-
Tetracycline	None	R(100%)	None	R(100%)	R(50%)	R(50%)	R(100%)	R(100%)	R(50%)	None	R(100%)	R(100%)
Nalidixic-acid	None	-	None	-	R(50%)	-	-	-	-	None	R(100%)	-
Ciprofloxacin	None	-	None	-	-	-	-	-	-	None	-	-

ZK, "Zoom-Koom"; EI, Edible Ice; -, None.

and 28.60%), nalidixic acid (13.90 and 14.30%), and amoxicillin-clavulanate (11.10 and 20.00%) and less resistant to chloramphenicol (8.30 and 8.60%), ceftriaxone (5.60 and 11.40%), cefamandole (5.60 and 8.60%), cephalothin (2.80 and 2.90%), gentamicin (2.80 and 5.70%), ciprofloxacin (2.80 and 2.90%), and cefepime (2.80 and 5.70%). Most EPEC strains are resistant to tetracycline, aztreonam and ticarcillin (Table 5).

DISCUSSION

The presence of *Salmonella* spp. in "zoom-koom" and edible ice samples indicates that consumers are exposed to infections. Our findings are relatively low than those reported in other studies on ice and fruits juice beverage (mango, orange etc) in Ivory Coast, Mexico and India (Agbessi et al., 2001; Falçao et al., 2002; Lewis et al., 2006). These data are not surprising because "zoom-koom" and ice are processed mainly by handling operations and without pasteurization treatment. Street-vended foods and beverages dominated by hand intervention without adhering to good hygienic practices can lead to contamination (Agbessi et al., 2001; Barro et al., 2002; Lewis et al., 2006; Elmahmood and Doughari, 2007; Sunday et al., 2011).

In our study, contamination of "zoom-koom" and edible ice caused by *E. coli* could be attributed to poor quality water and unhygienic practices during the production process. According to Makut et al. (2013), it is very possible that the pathogenic contamination could occur during hawking and improper or careless handling and packaging of the products. Indeed, Taylor et al. (2000) proved that the transfer of microorganisms to hands was due to poor personal hygiene after visiting the toilet. Our results on the presence of *E. coli* are in agreement with some of the earlier reports on its presence in fruit juices, soft drinks and ice in many developing countries (Barro et al., 2002; Falçao et al., 2002; Lateef et al., 2006; Lewis et al., 2006; Mahale et al., 2008; Uma Reddy et al., 2009; Sunday et al., 2011; Poojara and Krishna, 2012).

Regarding traditional beverages sold in markets, packaging inside a bag or recycled bottle requires a transfer. This manual transfer is sometimes done in the open air which causes the influx of flies that are likely to contaminate the product. In addition, a previously study conducted in Burkina showed that the flies landing on food or the surface of equipment can also spread bacteria, because they have contact with dirty matters (Barro et al., 2006).

Enteropathogenic *E. coli* (EPEC) serotypes, identified in our samples ("zoom-koom" and ice), cause severe foodborne disease (Stanilova et al., 2011). Indeed, it was reported that EPEC is transmitted by the fecal–oral route and is the major cause of infantile diarrhoea in developing countries (Clarke et al., 2002). According to Norazah et al. (1998) in Malaysia, EPEC presence in food indicated fecal contamination consecutive to unhygienic practices. The presence of EPEC in traditional drinks and edible ice could pose serious threats to the health of many consumers, like the "zoom-koom" and ice, which are highly consumed in Ouagadougou City. The markets are highly congested with people as a result, a single source of EPEC contamination could have widespread repercussion on people's health.

The rate of resistance obtained with antimicrobials tested is lower than those reported by Lateef et al. (2004) in orange juice in Nigeria and Lateef et al. (2006) in edible ice. This resistance of *E. coli* to beta-lactamin (amoxicillin-clavulanate, ticarcillin, ceftriaxone) could be explained by the production of beta-lactamase which hydrolyzes these antibiotics or the reduction of the structure of porins (Schwarz and Chaslus-Dancla, 2001). Also, the resistance to aminoglycosids such as gentamicin could be due to enzymatic activation by N-acetyltransferase and O-adenylyltransferase (Schwarz and Chaslus-Dancla, 2001). According to Lateef et al. (2004), the relatively high level of resistance to antimicrobial agents is a reflection of the misuse or abuse of these agents in the environment. The indiscriminate use of antibiotics, which promotes antibiotic resistance results from patients' demand, prescribers and dispensing doctors (Chinedum, 2005). Antimicrobial resistant strains of *Salmonella* spp. and *E. coli* isolated in this study constitute a serious public health problem.

From this study, it appears that "zoom-koom" and edible ice undergo fecal contamination during the various manufacturing processes. The presence of Enteropathogenic *E. coli* and *Salmonella* spp. strains in soft drinks and edible ice remains a significant public health concern. These pathogenic organisms have also shown to be highly resistant to antimicrobials tested, indicating a possible cause of public health hazards. Indeed, the ingestion of "zoom-koom" and ice contaminated with EPEC strains may cause the colonization of intestinal tract by antimicrobial resistant strains. Immediate action should be taken to spread awareness amongst the vendors about soft drinks safety and good hygienic practices in order to prevent contaminations and avoid any future pathogen outbreaks.

Conflict of Interests

The authors have not declared any conflict of interests.

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