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Antibiotic resistance in microbes from street hot beverages and vendors hygiene behavior in Abidjan, Côte d'Ivoire

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Abstract

Currently, the sale and consumption of hot beverages are increasing in Côte d'Ivoire streets, and the health risk assessment is still not well documented. It was then important to assess the health risks associated with potentially pathogenic microorganisms in these beverages, as well as their antibioresistance in the context of food safety. For this purpose, a survey including 500 hot beverage consumers was carried out in Abidjan streets. A total of 600 samples, including coffee (150), tea (150), milk (150), and cocoa (150), were aseptically collected from five locations in Abidjan city to determine their microbiological quality. The microorganisms isolated from beverages were identified using MALDI-TOF MS. A low prevalence of *S. aureus* (3.0%), *E. coli* (2.3%), *K. pneumoniae* (1.5%), *B. cereus* (1.3%), *C. tropicalis* (1.5%), and *C. parapsilosis* (1.0%) was detected in ready-to-drink beverage samples. Moreover, some microbial isolates were variously resistant to the antibiotic used in this study. Indeed, *S. aureus* was resistant to erythromycin, *K. pneumoniae* to piperacillin, *B. cereus* to penicillin, ampicillin and *C. tropicalis* to fluconazole. The presence of such antibiotic resistant strains constitutes a real risk health factor. Moreover, the survey on hot beverage street sellers and consumers showed that the conditions of hot beverages processing as well as materials handling, and seller's hygiene can be sources of contamination of pathogenic microorganisms. Street hot beverage consumers and sellers must improve hygienic conditions to prevent cross-contamination from human handling during food processing.

Keywords : Street hot beverages, potentially pathogenic microorganisms, antibiotic resistance

Résumé

Actuellement, la vente et la consommation de boissons chaudes augmentent dans les rues de Côte d'Ivoire, mais l'évaluation des risques sanitaires est encore mal documentée. Il est alors important d'évaluer les risques sanitaires associés aux micro-organismes potentiellement pathogènes présents dans ces boissons, ainsi que leur antibiorésistance dans le cadre de la sécurité alimentaire. A cet effet, une enquête auprès de 500 consommateurs de boissons chaudes a été réalisée dans les rues d'Abidjan. Au total, 600 échantillons, composés de café (150), de thé (150), de lait (150) et de cacao (150), ont été prélevés de manière aseptique sur cinq sites de la ville d'Abidjan pour déterminer leur qualité microbiologique. Les micro-organismes isolés des boissons ont été identifiés à l'aide de MALDI-TOF MS. Une faible prévalence de *S. aureus* (3,0 %), *E. coli* (2,3 %), *K. pneumoniae* (1,5 %), *B. cereus (*1,3 %), *C. tropicalis* (1,5 %) et *C. parapsilosis (*1,0 %) a été détecté dans des échantillons de boissons prêtes à consommation. Les isolats microbiens étaient diversement résistants à l'antibiotique utilisé dans cette étude. En effet, *S. aureus* était résistant à l'érythromycine, *K. pneumoniae* à la pipéracilline, *B. cereus* à la pénicilline, à l'ampicilline et *C. tropicalis* au fluconazole. La présence de telles souches résistantes aux antibiotiques constitue un réel facteur de risque sanitaire. Par ailleurs, l'enquête auprès des vendeurs ambulants de boissons chaudes et des consommateurs a montré que les conditions de transformation des boissons chaudes ainsi que la manutention des matériaux et l'hygiène des vendeurs peuvent être des sources de contamination par des microorganismes pathogènes. Les consommateurs et les vendeurs de boissons chaudes de rue doivent améliorer les conditions d'hygiène pour éviter la contamination croisée due à la manipulation humaine lors de la transformation des aliments.

Mots clés : Boissons chaudes, microorganismes potentiellement pathogènes, antibiorésistance

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Introduction

Street foods are ready-to-eat food or drinks (beverages) sold either on streets, in a market, park, or other public places. These foods are sold by a vendor from a portable food booth, food cart, or a portable stall, cart, or food truck meant for immediate consumption (Kok and Balkaran, 2014; Wiatrowski et al., 2021). Street foods account for a significant portion of urban food consumption for millions of low- and middle-income consumers every day. Approximately 2.5 billion people around the world consume street food every day (Abrar and Mohammedsani, 2020). Among these street drinks, hot beverages are consumed in diverse cities in Côte d'Ivoire specially in Abidjan the capital city (Atobla et al., 2020a). However, despite this high level of consumption and potential to contribute to food security, the conditions in which these hot beverages are sold exposed them to numerous contaminants that can compromise their safety. Such contamination was reported to be due to the vendors behavior and practices (Atobla et al., 2020b). Indeed, street foods can be cross-contaminated from various sources such as utensils, knives, raw foodstuffs, flies that sporadically land on the foods, vendors' bare hands serving, and occasional food handling by consumers (Tambekar et al., 2009). Consumers who depend on street food are more interested in its convenience and usually pay little attention to its safety, quality, and hygiene (Abrar and Mohammedsani, 2020). Wastewater and garbage are therefore discarded nearby, providing nutrients for insects and other household rodents, which may carry food-borne pathogens (Tambekar et al., 2009). Consequently, street food selling has become an important public health issue and a great concern for everybody (Rane, 2011). Various studies have identified the sources of food safety issues involved in street foods to be microorganisms belonging to *Escherichia coli*, *Pseudomonas aeruginosa*, Klebsiella, Bacillus, Staphylococcus, Clostridium, Vibrio, Campylobacter, Listeria, and Salmonella (Rane, 2011; Prabhakar and Gupta, 2018; Atobla et al., 2021). Street foods are perceived to be a major public health risk due to a lack of basic infrastructure and services and difficulty in controlling the large numbers of street food vending operations because of their diversity, mobility, and temporary nature (Ghosh et al., 2007; de Sousa, 2008). For example, *E. coli* has become a dangerous foodborne pathogen globally responsible for gastroenteritis epidemics in North America, Europe, Asia, and Africa, and many strains have been frequently implicated in undercooked foods, contaminated ground beef, raw milk, and unpasteurized cider (Hosein et al., 2008) and have the potential for the emergence of antibiotic resistance. The opportunistic pathogen *Bacillus cereus* is known to cause food-borne outbreaks in humans (Gao et al., 2018). *B. cereus* generally causes two types of gastrointestinal illness, including emesis and diarrhea, after consumption of contaminated food that contains more than 104–105 spores or vegetative cells of *B. cereus* per gram (Jensen et al., 2003; Bamnia and Kaul, 2015). The genera Saccharomyces, Aspergillus, Candida, and Penicillium were the eukaryotic microorganisms isolated in all beverages (Nwaiwu et al., 2020). *C. tropicalis, C. parapsilosis,* and *C. glabrata* are associated with 35% to 65% of all systemic Candida infections (Krcmery and Barnes, 2002), while C. krusei is fluconazole resistant (Cuomo et al., 2017). Infection with any non-albicans Candida strain increases patient morbidity, and coinfections with *C. tropicalis* and *C. glabrata* have high (40% to 70%) mortality rates (Krcmery and Barnes, 2002). Similarly, antibiotic resistance has been developed by microbes found in these foods. For example, resistance in *K. pneumoniae* has been observed in various community sources, including raw vegetables and ready-to-eat foods over the years, and there are published reports of *K. pneumoniae* having developed acquired resistance to carbapenems, the last-line drugs (Hartantyo et al., 2020; Giri et al., 2021). From different street foods at gazipur district (Bangladesh), *E. coli* isolates were resistant to ampicillin, penicillin G, chloramphenicol and cephalexin where sensitive to gentamicin and ciprofloxacin.

Klebsiella spp. isolates were resistant to ampicillin, cephalexin and sensitive to gentamicin, neomycin and ciprofloxacin. Gram positive *Staphylococcus spp.* were resistant to ampicillin, cephalexin and vancomycin and sensitive to were resistant to ampicillin, vancomycin, and cephalexin and sensitive to gentamicin and ciprofloxacin (Abdul et al., 2021).

Data on the sanitary conditions of street-vended hot beverages in Abidjan, as well as microbiological food quality and antibiotic resistance, are still scarce. Therefore, the objective of our study was to assess health risks associated with pathogenic microorganisms in street hot beverages and their microbial resistance to ensure consumers safety.

Material and Methods

Sampling Procedure

Hot beverages made of coffee, tea, milk, or cocoa were collected on the streets in five towns (Abobo, Adjamé, Yopougon, Cocody, and Port-Bouët) of Abidjan city, according to the method of Atobla et al. (2020a). This study was conducted from July 17th to December 19th, 2020.

Survey description

This survey was conducted among 500 people consuming hot street drinks sold on coffee carts. In Côte d'Ivoire, it is not necessary to receive written consent from participants for the structured written questionnaire. This survey was conducted among 500 people consuming hot beverages from street vendors with coffee carts. The respondents for these analyses were about 500 consumers who volunteered to respond to the questions. The Ethics Statement included in the questionnaire instructions clearly stated that only respondents who agreed to the instructions participated in the survey. All of the participants approved the statement before participating in the survey. The questions were only asked of those who reported consuming hot beverages from street vendors with push-hand mobile coffee carts.

Microbial isolation

In this study, *S. aureus*, *K. pneumonia*, *B. cereus*, Salmonella and yeast were isolated from 600 samples, composed of coffee (150), tea (150), milk (150), and cocoa (150). *S. aureus*, Chapman agar (Bio-Rad) was used according to standard NF V 08014:1984. The dishes were seeded and incubated in an oven at 37 °C for 24 hours. The appearance of small colonies surrounding a yellow halo or small colonies could indicate the presence of Staphylococcus. For confirmation of *S. aureus*, microscopic observation, catalase, DNase, and coagulase tests were performed. *S. aureus* ATCC 25923, a reference strain, was used as a positive control.

E. coli and *K. pneumoniae* isolation was carried out on Violet Red Neutral Bile Glucose (VRBG) agar (Bio-Rad) according to ISO standard 21528-2: 2004. These dishes were subsequently incubated at 37°C for 24 hours. The presence of enterobacteria was indicated by the presence of small, pink to red or colorless colonies (0.5 mm). *E. coli* and *K. pneumoniae* isolates were confirmed by MALDI-TOF mass spectrometry at the biobank laboratory of the Pasteur Institute of Côte d'Ivoire.

B. cereus was isolated using mannitol egg yolk polymyxin (MYP) agar plate. A serial dilution was prepared for the enumeration of *B. cereus*. At the end of each dilution step, 0.1 mL of the inoculum was aseptically spread on plate. The inoculated agar plates were then incubated at 37°C for 24 h. Following the incubation period, all agar plates were examined for potential *B. cereus* colonies and enumerated. The bacterial count was based on all presumptive colonies on the selective agar medium (MYP). On MYP agar, presumptive *B. cereus* colonies appeared as rough and dry cultures with a diameter of 2 –5 mm. Typical colonies growing on MYP agar plates could thus be identified as *B. cereus*. After a microscopic observation (Gram-positive, rod-shaped, motile) was carried out, Bacillus colonies were purified on nutritive agar.

Salmonella isolation was conducted following the ISO 6579 standard protocols (ISO 6579, 2002). Briefly, 10 ml of beverage samples were put in 90 ml of buffered peptone water (Bio-Rad, Marnes-la-Coquette, France) as a pre-enrichment medium, then 0.1 ml of culture broth was added to 10 ml of Rappaport Vassiliadis as a selective enrichment medium, and 0.1 ml of inoculum was spread on Hektoen agar and on XLD agar (Bio-Rad, Marnes-la-Coquette, France) as selective medium.

Yeasts isolation was done using Sabouraud Chloramphenicol agar (SCA, Biokar Diagnostics, France). According to the standards' specifications, 0.1 mL of beverage sample is aseptically transferred to Petri dishes. Yeast enumeration was carried out according to the NF/ISO 16212: 2011 standard. All the Petri dishes were then incubated in an oven at 30 °C for 48 hours for the enumeration of yeasts. The appearance of white to yellowish colonies would indicate the presence of yeasts. Colonies identified as yeasts by their macroscopic aspects and their microscopic observations in the fresh state were purified by striae on Sabouraud agar.

Microbial identification using MALDI-TOF MS

Microorganisms were confirmed by MALDI-TOF mass spectrometry at the biobank laboratory of the Pasteur Institute of Côte d'Ivoire. The confirmation of microorganisms by borrowing the mass of ribosomal proteins was done by the MALDI-TOF (Vitek MS BioMerieux, France) mass spectrometer, which is a spectrometer using a matrix-assisted laser ionization source and a time-of -flight analyzer. MALDI-TOF identification is done in three steps: sample preparation, followed by sample analysis, and data processing (Lo et al., 2017). A colony of the calibration strain, Escherichia coli ATCC 8739, was spotted onto a MALDI-TOF plate with 1 µL of CHCA matrix (α-cyano-4-hydroxycinnamic acid, MS CHCA ref 411071). Sterile loop samples from each colony were then deposited in target wells for testing in duplicate. For yeasts, 0.5 µL of formic acid (Vitek MS-FA, ref 411071) was added to each well. After air-drying (approximately 5 min), 1 µL of the matrix was added to each spot, and they were again dried. Once this was done, the slide was inserted into the Vitek MS, and analysis was instigated after transferring the data from the Prep Station to the Vitek MS. Sample preparation was performed using the Prep Station, a module consisting of a computer and a barcode reader, which were used to enter the various sample data and their sites onto the slide. Measurements were performed with the MALDI BioTyper MYLA® software and the spectra obtained were compared with those from the database for validation. The results were measured by two parameters, namely the degree of confidence, or percentage score, and the confidence level of the different colors. Green with a score between 99.9 and 60% indicates good identification, orange with a score of < 60% indicates a low probability of identification, and red with a score of zero indicates no identification.

Microbial antibiotic susceptibility

S. aureus antibiotic susceptibilities were determined by the disc diffusion method on Mueller-Hinton agar (Oxoid, France) according to the National Committee for Clinical Laboratory Standards (NCCLS, 2000). The plates were incubated for 24 hours at 37°C and resistance was scored via visual examination. The antimicrobial drugs tested and their concentrations on the discs (Bio-Rad, Marnes-La-Coquette, France) were as follows: penicillin (PEN, 10 µg), amoxicillin (AMX, 25 µg), cefoxitin (FOX, 30 µg), tobramycin (TM, 10 µg), gentamicin (GMN, 15 µg), erythromycin (ERY, 15 µg), clindamycin (CMN, 2 µg), levofloxacin (LVX, 5 µg), norfloxacin (NOR, 5 µg), ofloxacin (OFX, 5 µg), and vancomycin (VAN, 30 µg). As a test control, Staphylococcus aureus ATCC 25923 was used. Isolates were classified as susceptible or resistant to the drug.

For *K. pneumoniae,* the tested antibiotics and their concentrations on the discs (Bio-Rad, Marnes-La-Coquette, France) were as follows: amoxicillin (AMX, 20 µg), amoxicillin-clavulanic acid (AMC, 20-10 µg), ticarcillin (TIC, 75 µg), piperacillin (PIP, 30 µg), piperacillin-tazobactam, (TZP, 30/6 µg), cefoxitin (FOX, 30 µg), cefepime (FEP, 30 µg), ceftriaxone (CRO, 30 µg), imipenem (IPM, 10 µg), amikacin (AKM, 30 µg), gentamicin (GMN, 10 µg), levofloxacin (LVX, 5 µg), nalidixic acid (NA, 30 µg), and tigecycline (TCG, 15µg).

Bacillus strains was evaluated by the Kirby–Bauer disk diffusion method according to the performance standards for antimicrobial susceptibility testing of the Clinical and Laboratory Standards Institute (the Clinical and Laboratory Standards Institute). Fifteen antibiotics (Oxoid, United Kingdom) were tested, including ampicillin (AMP, 10 µg), amoxicillin-clavulanic acid (AMC, 20 µg/10 µg), penicillin (P, 10 U), cephalothin (KF, 30 µg), cefoxitin (FOX, 30 µg), imipenem (IPM, 10 µg), gentamicin (GM, 10 µg), kanamycin (K, 30 µg), erythromycin (E, 15 µg), vancomycin (VA, 30 µg), ciprofloxacin (CIP, 5 µg), chloramphenicol (C, 30 µg), tetracycline (TE, 30 µg), trimethoprim -sulfamethoxazole (SXT, 1.25 µg/23.75 µg), and clindamycin (2 µg). After incubation for 24 h at 37ºC, inhibition zones were measured and interpreted referring to the zone diameter interpretive criteria. These antibiotics are commonly applied as an antibiotic agent in the treatment of Bacillus infections.

Pathogenic yeasts to antifungal drugs susceptibility testing were performed for isolates of Candida species by using ATB Fungus 3® from Biomérieux as described by Eddouzi et al. (2013). This method enables determining the susceptibility of Candida species isolates to the antifungal agents in a semi-solid medium following the conditions recommended by the European Committee on Antibiotic Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI). ATB Fungus 3® was performed following the manufacturer's instructions. Briefly, Mueller-Hinton agar supplemented with 2% of glucose and 0.5 µg/ml of methylene blue was prepared in plates. Each isolate was cultured overnight at 30 °C for 48 hours to ensure purity and viability. The inoculum was adjusted to the turbidity of a 0.5 McFarland standard in sterile saline (0.85%) and streaked onto plates by using a cotton swab. Aseptically, fluconazole (FLC, 25 µg) and voriconazole (VRC, 1 µg) disks (Becton Dickinson, Sparks, MD, USA) were placed on the agar surface. The inoculated strips were read visually after incubation at 30°C for 48 h. The results obtained gave the minimum inhibitory concentration (MIC) that helps to classify the strain as insensitive, intermediate, or resistant. The MIC interpretive guidelines for in vitro susceptibility were tested for Candida species. All experiments were performed in duplicate.

Estimation of risk factors for street hot beverage activities

The potential risk factors that could justify the level of contamination of the samples and the symptoms

reported during the survey were listed based on direct observations and statements made by consumers.

Data analysis

The data obtained from the questionnaire and observation checklists were analyzed using IBM SPSS version 25.0 and tabulated in Microsoft Excel. Descriptive statistics were used to summarize the variables of interest and determine relationships between them. The results were expressed as mean ± standard deviations (±SD), frequencies, and percentages. The Chi-square test was used to test then relationships between the variables. The difference between the variables was considered significant at $p < 0.05$.

Results

Prevalence of potential pathogenic microorganisms

Tea was the beverage most contaminated by *S. aureus* (7.3%). Also, tea was more contaminated with *E. coli* (4.6%) than *K. pneumoniae* (3.3%). *B. cereus* was found in hot beverage samples (1.3%). The contamination due to *B. cereus* was 2.6% (4/150) in milk and 3% (3/150) in tea. In contrast, no contamination of *B. cereus* was detected in coffee (Table 1).

C. tropicalis (3.3%) and *C. parapsilosis* (2%) were isolated mostly in tea. Coffee was the least contaminated beverage (3.3%), followed by cocoa (5.3%). Of the 600 beverage samples evaluated, 64 (10.6%) samples contained potential pathogenic microorganisms (Table 1).

Salmonella and *Candida albicans* were not found in the tested beverage samples. *S. aureus*, *E. coli* and *C. tropicalis* were mostly found in tea. Coffee was the least contaminated beverage (Table 1).

Distribution of pathogenic resistance to antibiotics

All the *S. aureus* isolated from milk were resistant to erythromycin (Table 2).

Table 2: Distribution of *S. aureus* antibiotic resistance

All *K. pneumoniae* isolated from beverages were resistant to piperacillin but sensitive to imipenem and amikacin. Coffee´s *K. pneumoniae* was more resistant than tea, milk or cocoa´s *K. pneumoniae* (Table 3).

All E. coli isolates from tea were resistant to piperacillin (100%). There was no resistant *E.* coli found with antibiotics such as cefepime, imipenem, amikacin, gentamicin, levofloxacin, and tigecycline. All *B. cereus* isolates were tested for antimicrobial susceptibilities to 15 selected antibiotics.

It was shown that all *B. cereus* isolates were resistant to penicillin (PEN, 100) and ampicillin (AMP, 100%), amoxicillin-clavulanic acid (AMC, 88.8%), cephalothin (KF, 77.8%), and cefoxitin (FOX, 88.9%), which belong to β-lactams. All isolates were susceptible to antibiotics, such as imipenem (IPM; 0%), gentamicin (GEN, 0%), ciprofloxacin (CIP, 0 %), and chloramphenicol (C, 0%).

Table 1: Distribution of potential pathogenic bacteria and yeasts

Hot beverage items	Total examined	Pathogenic bacteria isolates						Total N (%)
		S. aureus n (%)	*E. coli* n (%)	*K. pneumoniae* n (%)	*B. cereus* n (%)	*C. tropicalis* n (%)	*C. parapsilosis* n (%)	
Coffee	150	1 (0.6)	1 (0.6)	1 (0.6)	0 (0)	1 (0.6)	1 (0.6)	5 (3.3)
Tea	150	11 (7.3)	7 (4.6)	5 (3.3)	3 (2.0)	5 (3.3)	3 (2.0)	34 (22.6)
Milk	150	4 (2.6)	4 (2.6)	2 (2.0)	4 (2.6)	1 (0.6)	2 (1.3)	17 (11.3)
Cocoa	150	2 (1.3)	2 (1.3)	1 (0.6)	1 (0.6)	2 (1.3)	0 (0.0)	8 (5.3)
Total	600	18 (3.0)	14 (2.3)	9 (1.5)	8 (1.3)	9 (1.5)	6 (1.0)	64 (10.6)

Table 2: Distribution of *S. aureus* antibiotic resistance

Antibiotic families	Antibiotics	Coffee' *S. aureus* (n=1)	Tea' *S. aureus* (n=11)	Milk' *S. aureus* (n=4)	Cocoa' *S. aureus* (n=2)	Total of beverage' *S. aureus* n=18 (100%)
Penicillins	PEN (10µg)	1	6	3	2	12 (66.7)
	AMX (25 µg)	0	3	1	2	6 (33.3)
Cephalosporin	FOX (30 µg)	0	0	0	0	0 (0.0)
Aminosides	TM (10 µg)	1	0	1	0	2 (11.1)
	GM (10 µg)	1	0	1	0	2 (11.1)
Macrolides	ERY (15 µg)	1	11	4	2	18 (100)
	CMN (2 µg)	1	1	2	1	5 (27.8)
Quinolones	NOR (5 µg)	0	1	2	0	3 (16.6)
	OFX (5 µg)	0	1	2	1	4 (22.2)
	LVX (5 µg)	0	1	1	1	3 (16.6)
Glycopeptides	VAN (30 µg)	0	0	0	0	0 (0.0)

Table 3: Factors favoring the consumption of street hot beverages

Consumption reasons	Number of surveys	Frequency (%)
Cost	425	85
Taste	345	69
Convenience	330	66
Religion	205	41
Culture	170	43

C. tropicalis was more resistant to fluconazole (44.4%) than voriconazole (0.0%). Moreover, only one C. parapsilosis isolate in tea was resistant to the azole drugs. No Candida species was resistant to Voriconazole but, two C. parapsilosis (12.5%) were resistant to 5-Flucytosine.

Factors influencing street hot beverage consumption and associated risks

The factors that contributed to why people prefer drinking street hot beverages to other foods in Abidjan city were the low cost (85%). The survey also found that most of the respondents chose to drink hot beverages because of their taste (69%) or convenience due to time constraints (66%) of hot beverages (Table 3)

The handling and adding of ingredients (sugar, lemon, etc.) could cause cross-contamination due to the handling or hygiene of street vendors. Homogenization of the mixture in a recyclable cup or improperly disposable cups could be source of contamination by microorganisms in the environment.

The factors that should be considered for analyzing the hazards due to street hot beverages are many. The sources (vendor location, personal hygiene, materials, ingredients), the type of hazard (improper handling), and the microbial risk involved (transmission or cross-contamination of microorganisms) in street hot beverages are reported in Table 4.

During street hot beverage processing, some contaminants could occur related to the process. The highest factor risks were recorded through the handling of products, materials, ingredients, or water used to prepare and rinse recyclable cups. In addition, a supplement contamination factor is the recyclable cups used to homogenize the mixture. The cooking process is also one of the determining factors linked to beverage poisoning risks. Hot beverage contamination varied according to several factors (Table 5).

Table 4: Probable risks related to hot street beverage consumption

Categories	Theme	Contaminant level
Hot beverages	Coffee, milk, coffee with milk, cocoa	-
	Tea with ingredients	++
Ingredients	Sugar, lemon, or mint	+
Materials	Thermos for hot water, spoon, knife, scissors	+
	Lemon squeezer	++
	Water used to prepare and rinse the recyclable cups	++
Other risks	Disposable cups	+
	Recyclable cups	++
	Handling of products, materials, or ingredients	+++
	Beverage cooking process	++
	Hygiene of vendors	+

- absence of risk, + low risk, ++ moderate risk, +++ high risk.

Table 5: Street hot beverages contamination by potential microorganisms

Pathogenic isolates	Coffee	Tea	Milk	Cocoa
Staphylococcus aureus	-	++	+	+
E. coli	-	++	+	+
K. pneumoniae	+	+	+	+
Bacillus cereus	-	+	+	+
Salmonella	-	-	-	-
Candida tropicalis	+	++	+	+
Candida parapsilosis	+	+	+	+
Candida albicans	-	-	-	-

-, absence; +, low contamination; ++, moderate contamination; +++ high contamination

Discussion

In Côte d'Ivoire, the sale and consumption of hot beverages are increasing on the street, despite the conditions of handling, preparation, and sale of street beverage vendors, which can expose them to numerous contaminations. These contaminations may be due to the behavior and practices of these vendors, to the preparation methods, or to the characteristics of the sale regarding hygiene and safety, which leave something to be desired (Atobla et al., 2020b).

Thus, the main factors that contributed to why people prefer drinking street hot beverages to other foods in Abidjan cities in this study were the low cost (85%), taste (69%), or convenience due to time constraints (66%) of hot beverages. For consumers, elements such as practicality, accessibility, variety of options, appreciable palatability, and affordable price are attractive, considering the moment of choice for this kind of meal (Gupta et al., 2018; Soon, 2019). According to Imathiu (2017), the kinds of food sold on the streets vary according to local culture, taste, and customer preference. Mathye and Maliwichi (2015) revealed that most of the respondents (80%) chose to eat street foods because of the low cost of the meals compared to the cost of other foods. Besides, the consumption of hot beverages surveyed is the same as that of those in Africa, Asia, and South America, where it is based on low-priced food (da Silva et al., 2014; Choudhury et al., 2011; Wiatrowski et al., 2021).

The handling of ingredients (sugar, lemon, or mint) could cause cross-contamination due to the handling of raw materials or the hygiene of vendors. According to previous studies on street food in many countries (da Silva et al., 2014; Azanza et al., 2000; Atobla et al., 2021; Wiatrowski et al., 2021), food production is associated with poor hygiene and distribution, which poses a hazard to the health of consumers. Many of these street food trades have empirical handling practices, poor hygiene practices, inadequate infrastructure, and a lack of potable water points (Amare et al., 2019; Chávez-Martinez et al., 2019; Garayoa et al., 2017). Therefore, street foods cause great concern among agencies involved in food safety (Trafialek et al., 2017) and are generally associated with foodborne illnesses, which are known to be an important global public health problem (Chávez-Martinez et al., 2019; Trafialek et al., 2018; Soon, 2019). Besides, street food is strongly associated with the lack of hygienic and sanitary control in food production processes, such as hand hygiene and handling (Abrahale et al., 2019; Mali et al., 2019; Tomar and Akarca, 2019). Also, the homogenization of the mixture in a recyclable cup can be a source of contamination by microorganisms in the environment. Thus, this practice clearly shows that most of traders do not pay attention to the prevention of cross-contamination between foods (Ferrari et al., 2021). According to Proietti et al. (2014), there is a high risk of environmental contamination due to the absence of running water or potable water storage. Also, ready-to-eat food can easily be contaminated again because of general poor hygiene, such as unclean preparation sites or cleaning utensils, cross-contamination, and inadequate personal hygiene (Niyonzima et al., 2017).

In this study, homogenization of the mixture in a recyclable cup or improperly disposable cup could be a source of contamination by microorganisms in the environment. In Nigeria, according to Nwaiwu et al. (2020), the packaging could also be compromised because many producers sell their products in used plastics that may not be sanitary. Factors such as improper storage temperature of the water, insufficient heating, hot water cooling, or time of water storage could constitute a hazard, resulting in microbial risks. Tambekar et al. (2009) reported that time of fruit preparation, hygiene of vendors, and the area surrounding vending sites are other important factors. In fact, tea was the beverage most contaminated by S. aureus (7.3%). Also, tea was more contaminated with *E. coli* (4.6%) than *K. pneumoniae* (3.3%). Bacillus cereus was found in hot beverage samples (1.3%). The contamination of *B. cereus* was 2.6% (4/150) in milk and 3% (3/150) in tea. In contrast, no contamination of *B. cereus* was detected in coffee. *C. tropicalis* (3.3%) and *C. parapsilosis* (2%) were isolated mostly in tea. Coffee was the least contaminated beverage (3.3%), followed by cocoa (5.3%). Of the 600 beverage samples evaluated, 64 (10.6%) samples contained potential pathogenic microorganisms as reported in Table 1. Other authors also reported a relatively low percentage (14%) of street food vendors with high levels of

compliance regarding sanitation (Nkosi and Tabit, 2021; Wiatrowski et al., 2021). In their studies, Nwaiwu et al. (2020) found a low prevalence of bacteria associated with hygiene, especially the Escherichia genus in alcoholic beverages such as palm wine, pito, and burukutu, which may be due both to low acidity and high ethanol content. Various studies have also identified the sources of food safety issues, involved in street foods to be microorganisms belonging to the genus Bacillus, Staphylococcus, Clostridium, Vibrio, Campylobacter, Listeria, Salmonella (Rane, 2011; Prabhakar and Gupta, 2018; Atobla et al., 2021). According to Paudyal et al. (2017), the microorganisms most commonly found in contaminated foods are *E. coli,* Salmonella spp., and *S. aureus*. *S. aureus* is a microorganism that inhabits the skin, hands, respiratory region, and superficial wounds and is, therefore, an important indicator of hygiene behavior (Adjrah et al., 2013; Tomar and Akarca, 2019). Getu et al. (2013) revealed in their work that poor personal hygiene, improper handling, and storage practices of foods, and poor knowledge of food vendors towards foodborne diseases were the associated risk factors for contamination of street foods in Gondar. The food handler can cause contamination, as the hands are important vehicles for the transfer of organisms from the feces, nose, and skin to the food (Loukieh et al., 2018; Amare et al., 2019). In this study, *B. cereus* was found in hot beverage samples (1.3%). Intriguingly, the overall contamination level of B. cereus is lower, indicating that *B. cereus* risk in street hot beverages can be serious. Besides, the conditions for milk production, handling, and processing could introduce *B. cereus* into dairy products (Cui et al., 2016; Kumari and Sarkar, 2016).

All the S. aureus isolated from milk were resistant to erythromycin (Table 2), while all *K. pneumoniae* isolated from beverages and *E. coli* from tea were resistant to piperacillin but sensitive to imipenem and amikacin. It was shown that all E. coli isolates from tea were resistant to piperacillin. There was no resistant *E. coli* found in antibiotics such as cefepime, imipenem, amikacin, gentamicin, levofloxacin, and tigecycline (Table 4). *E. coli* has become a dangerous foodborne pathogen globally responsible for gastroenteritis epidemics in North America, Europe, Asia, and Africa, and many strains have been frequently implicated in undercooked foods, contaminated ground beef, raw milk, unpasteurized cider and apple juice, bean sprouts, or fresh leafy vegetables such as lettuce and spinach (Hosein et al., 2008), and have a potential for the emergence of antibiotic resistance. Similarly, resistance in *K. pneumoniae* has been observed in various community sources, including raw vegetables and ready-to-eat foods over the years, and there are published reports of *K. pneumoniae* having developed acquired resistance to carbapenems, the last-line drugs (Hartantyo et al., 2020; Giri et al., 2021). Bacteria such as *B. cereus* may cause severe diseases and infections that even lead to death (Lund et al., 2000; Dierick et al., 2005). In our study, B. cereus isolates were more resistant to β-lactam antibiotics but susceptible to carbapenems (imipenem), quinolones (ciprofloxacin), aminoglycosides (gentamicin), and amphenicols (chloramphenicol). *B. cereus* was resistant to β-lactam antibiotics, which was consistent with previous studies (Fernandes et al., 2014; Kim et al., 2015; Yibar et al., 2017). For Gao et al. (2018), *B. cereus* isolates that display resistance to three or more antibiotics should be given more attention. In this study, only one *C. parapsilosis* isolate in tea was resistant to the azole drugs. Moreover, examples of resistance to well-known antifungal drugs have been reported; e.g., *C. glabrata* is particularly prone to developing resistance to fluconazole, a first-line antifungal treatment for yeast infections (Abbes et al., 2011), and resistance to fluconazole and other azoles appears to be increasing among clinical isolates of *C. tropicalis* (Pfaller and Castanheira, 2016). Consequently, further research is needed to optimize fermentation conditions to eliminate opportunistic pathogenic yeasts during processing, and equally

important, improved hygienic conditions need to be ensured in order to prevent cross-contamination from human handling during food processing. In our work, *C. tropicalis* was more resistant to fluconazole than voriconazole. In the study by Eddouzi et al. (2013), the in vitro susceptibility of Candida species showed that the incidence of fluconazole resistance was higher than for voriconazole. Despite the increase in the frequency of fungal infections caused by the yeast C. tropicalis, azole resistance has been insufficiently investigated. Furthermore, *C. tropicalis* develops drug resistance more rapidly in the presence of Fluconazole than other Candida species (Barchiesi et al., 2000; Eddouzi et al., 2013).

The factors that should be considered for analyzing the hazards due to street hot beverages are the source (vendor location, personal hygiene, materials, ingredients), the type of hazard (improper handling), and the microbial risk involved (transmission or cross contamination of microorganisms). de Sousa (2008) pointed out that street foods are perceived to be a major public health risk due to the lack of basic infrastructure and services and the difficulty in controlling the large numbers of street food vending operations. Before, during, and after food preparation, good cleanliness measures can lower the risk of developing an infection (Normanno et al., 2007). *E. coli* is considered an important enterobacterium. Its presence in food is used as an indicator of fecal contamination and low hygienic conditions in food handling, especially hand hygiene, cross-contamination through utensils, and contact between raw and ready-for-consumption materials. For that reason, it should not be present in ready-to-eat foods (Shiningeni et al., 2019).

Both yeasts most probably occur because of contaminations during processing due to improper human handling. Contrary to this, *C. tropicalis* is frequently identified in indigenous sub-Saharan African fermented foods and beverages. Candida spp. have emerged as major agents of human mucosal, systemic, and bloodstream yeast infections (Silva et al., 2012; Pfaller and Castanheira, 2016). Other studies have also pointed out significant failures in the application of good handling practices among street vendors (Adimasu et al., 2016; Proietti et al., 2014; Samapundo et al., 2015, 2016; Trafialek et al., 2017). One of the most important steps in preventing food contamination and reducing or eliminating possible food pathogens, is the adequate hygienic practice of the food handler (Tomar and Akarca, 2019). Therefore, this result revealed the low exposure of hot beverages to contamination and the increased risk of the consumer acquiring foodborne diseases. Wiatrowski et al. (2021) report similar results. The reason for the bad hygienic conditions in Abidjan's street vendors may be the education of street food vendors, where only a small percentage of vendors have secondary education (10.7%), primary (17.3%) and the rest are illiterate (Atobla et al., 2020b). However, some authors suggest that it is not the level of education, but rather training and licensing, that have the effect of increasing food safety knowledge and improving food handling practices (Nyoni and Bonga, 2019). It is also important that the vendors take care of their personal hygiene and adopt appropriate food handling behaviors. This is especially important because food handlers can cause cross-contamination between ingredients and ready-to-drink beverages, and they may compromise food hygiene through improper preparation, cooking, and storage of foods (Walker et al., 2003). In the study by Atobla et al. (2020a), the authors reported that 5.6% of hot beverage consumers surveyed had negative effects such as diarrhea (1.2%), nausea (0.6%), vertigo (0.6%), and hand tremors (0.6%) after consuming hot beverages from street beverage vendors with coffee carts in Côte d´Ivoire. In this study, certain solutions were presented to address health hazards associated with street hot beverage consumption, such as a surveillance mechanism targeted at limiting the microorganisms that cause food poisoning. According to Normanno et al. (2007), improper food handling, preparation, or storage are the most common causes of foodborne diseases.

Before, during, and after food preparation, good cleanliness measures can lower the risk of developing an infection (Normanno et al., 2007). According to Okaru et al. (2019), unregulated beverages are commonly referred to as artisanal, unrecorded, illicit, or illegal drinks in a community. A common attribute of these kinds of products is that they are produced outside government regulation without any rules or regard to standard food safety guidelines. These kinds of products are generally untaxed by the government, and anybody can become a manufacturer (Nwaiwu et al., 2020).

In the open air, some sellers displayed their recyclable and disposable cups. Tambekar et al. (2009) reported that the location by the side of a busy road with heavy vehicular traffic (airborne particles) or by the side of the waste disposal system and overcrowding seems to add to the contamination. Such locations should be avoided for establishing street vending operations. Some recommendations to avoid contamination in street hot beverages were proposed to vendors, consumers, and public authorities in this study. Vendors must wash the preparation materials after using them or use clean water for washing equipment and preparing hot beverages. Kanaan (2013) recommended verifying a new novel strategy in the food chain through changing hazard analysis and critical control points (HACCP) strategies concerning the environmental epidemiology of MRSA populations from good management manufacturing practices to consumers with the aid of new methodologies. Mimicking the behavior of this pathogen would reduce the danger of contamination of food-products and cases of food poisoning in humans (Kanaan, 2013). It has been pointed out that beverages such as fruit juices, coffee, tea, and alcoholic beverages are likely to be targets of food fraud by adulteration through practices that may involve mixing or substituting the original components (Kamiloglu, 2019). Imathiu (2017) said that the current challenges that governments in developing countries need to tackle to ensure that the full potential of street foods is realized include: ensuring the safety of these foods by enlightening both consumers and vendors through training on best food-handling and processing practices to prevent foodborne diseases caused by both microbiological and chemical hazards.

Conclusion

To our knowledge, this is the first study to assess pathogenic microorganisms in hot beverages in Côte d'Ivoire. The present study has revealed that a low prevalence of pathogenic microorganisms is involved in street hot beverages. The presence of these opportunistic pathogenic microorganisms in street beverages could constitute a health concern, especially since the resulting beverages are consumed directly. Thus, hygienic conditions are necessary to eliminate opportunistic pathogenic microorganisms during processing, and equally important, improved hygienic conditions need to be ensured in order to prevent cross-contamination from human handling during food processing. Although beverages are consumed hot, the presence of pathogenic microorganisms and unsatisfactory hygienic conditions should challenge consumers as well as the authorities concerned in order to avoid any risk of infection.

Disclosure of interest

The author declares that he has no conflicts of interest concerning this article.

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