

Antibiotic Resistance and Virulence Genes of *Escherichia coli* and *Klebsiella* sp. Isolated from hot beverages sold on the streets of Abidjan, Côte d'Ivoire

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Abstract

Background: The presence of enterobacteria indicative of fecal contamination in street foods is becoming increasingly worrying, especially in hot beverages. The objective of this work was to assess the risk of these street beverages due to the presence of *Escherichia coli* and *Klebsiella* sp. strains producing extended-spectrum beta-lactamases (ESBL) possessing virulence and antibiotic resistance genes.

Materials and Methods: Thus, the prevalence of these strains was determined by the method of biochemical identification. Antibiotic susceptibility tests and double disc synergy to detect ESBL were carried out. Antibiotic resistance genes such as the *bla*_{TEM}, *bla*_{SHV} and *bla*_{CTX} genes as well as the *sxt1*, *sxt2*, *eaeA* and *bfpA* virulence genes were searched by PCR method.

Results: The rates of *E. coli* (5%) and *Klebsiella* sp. (2.5%) were higher in tea. No strain of *E. coli* had produced extended-spectrum beta-lactamases and had resistance genes, but 1 (5%) strain had the virulence gene *sxt1* while two strains of *Klebsiella* sp. (6.7%) were producers of ESBL. Furthermore, all strains of *Klebsiella* sp. producing extended-spectrum beta-lactamases and one non-producing strain of ESBL possessed the beta-lactam resistance genes *bla*_{TEM} up to 30% and 20% to the *bla*_{SHV} genes. However, none of the strains harbored *bla*_{CTX-M}. *E. coli* and *Klebsiella* sp. were 100% resistant to piperacillin. *E. coli* was 100% sensitive to Cefepime, Amikacin, and Gentamicin.

Conclusion: The presence of *Escherichia coli* with the *sxt1* gene and *Klebsiella* producing ESBL are alerts for consumers because these beverages could be sources of disease.

Keywords: Hot beverages, *E. coli*, *Klebsiella*, antibiotic resistance, extended-spectrum beta-lactamase

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I. Introduction

Ready-to-eat foods are consumed in both developed and developing countries. They are widely used in Asia including Pakistan¹, Indonesia², Malaysia³ and Africa, such as in Ghana⁴, Benin⁵, Burkina Faso⁶, Nigeria⁷, Côte d'Ivoire^{8,9,10} and many other countries. Among these ready-to-eat foods, we have hot beverages. They are generally prepared from hot water or obtained by adding hot water to the base material, which can be coffee, tea, milk, or cocoa, and preferably add sugar or lemon for tea^{8,9,10}.

In Côte d'Ivoire, especially in Abidjan, street beverages are increasingly consumed by the population. Street hot beverage vending is a very flourishing activity, mainly developed by nationals of the sub-region, also called itinerant sellers⁸. However, despite their high consumption and their potential to contribute to the food security of the Ivorian population, the conditions under which these drinks are prepared and sold in the street with mobile carts expose them to numerous contaminants⁸. These contaminations are the result of these vendors' behaviors and practices; preparation methods; sales characteristics in terms of hygiene and health safety; and the water used^{8,9,11}. Some of the water comes from public toilets^{10,11,13}. The consequence of these inadequate practices is the presence of faecal contamination indicator microorganisms¹⁴ and transmission of foodborne diseases¹⁵. If the studies of Shinichi *et al.*¹⁶ have shown that the consumption of certain hot drinks such as coffee has a beneficial effect, it must be recognized that many studies have reported the role of street foods as vectors of pathogenic bacterial transmission to humans^{10,17} and these studies have highlighted the presence of potentially pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp., *Shigella* sp., total coliforms, *Bacillus* sp., *Klebsiella* sp., *Pseudomonas* sp., *Streptococcus* sp. and some filamentous fungi such as *Aspergillus* sp.^{3,4,5}. Some of these contaminants cause serious diseases like typhoid, dysentery, enteric fever, and peripheral as well as systemic mycoses, which represent threats to human health^{18,19} and constitute a public

health problem. Thus, foodborne diseases are an important cause of morbidity and mortality because millions of people fall ill and many die after ingesting food that is unfit for consumption^{8,20}.

The treatment of these infectious diseases due to these microorganisms isolated from street foods and particularly hot beverages requires the use of antibiotics. However, several studies^{17,20} have shown the resistance or even multi-resistance of germs isolated from ready-to-eat street foods^{10,21}. Furthermore, De Wals²² highlighted the therapeutic failures due to resistance to several antibiotics simultaneously.

The objective of this work was to assess the risk of these street beverages due to the presence of *Escherichia coli* and *Klebsiella* spp. possessing virulence and antibiotic resistance genes.

II. Materials and Methods

Materials

The material used in this study consisted of 76 presumptive *Enterobacteriaceae* collected during a previous study^{8,9} from hot beverages based on coffee, tea, and milk, collected from street vendors with carts.

Methods

Biochemical identification

This step consisted of highlighting the different strains by means of the specific biochemical tests using Le Minor's miniature gallery. This involved highlighting the production of cytochrome C oxidase, the use of carbon from citrate as the sole carbon source, the production of urease, indole, tryptophan deaminase, the production of hydrogen sulfide, gas, fermentation of lactose and glucose, lysine decarboxylase, lysine deaminase and motility determination, fermentation of mannitol, and the reduction of nitrates to nitrites. The strains of *E. coli* and *Klebsiella* were confirmed by MaldiTof as described by Atobla et al.¹².

E. coli and *Klebsiella* sp. Antibiotic susceptibility

The susceptibility of *E. coli* and *Klebsiella* spp. isolates to several antibiotics (Table n°1) was tested by the Mueller Hinton agar diffusion method according to the recommendations of European Committee on Antimicrobial Susceptibility Testing/ Antibiogram Committee of the French Society of Microbiology(2020) (EUCAST/CA-SFM). The dishes were then brought to the oven at 37°C under aerobiosis for 24 hours in an upside-down position. 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 (EUCAST/CA-SFM). Thus, an isolate may be susceptible (S) to an antimicrobial agent or intermediate (I) or resistant (R).

Table n° 1: Antibiotics tested

Class	Antibiotics	Disc load (µg)	Critical diameters (mm)	
			S ≥	R <
Penicillins	Ticarcillin	75	23	20
	Amoxicillin	20	19 ^{AB}	19 ^{AB}
	Piperacillin	30	20	17
	Piperacillin-tazobactam	30-6	20	17
	Amoxicillin-clavulamicacid	20-10	19 ^B	19 ^B
Cephalosporins	Cefoxitin	30	19	15
	Cefepime	30	27	24
	Ceftriaxone	30	25	22
Carbapenems	Imipenem	10	22	17
Aminosides	Amikacin	30	18	18
	Gentamicin	10	17	17
Fluoroquinolones	Levofloxacin	5	23	19
	Nalidixicacid	30	14	14
Tetracyclines	Tigecycline	15	18 ^A	18 ^A

A: Field strains of group I Enterobacteriaceae (*E. coli*, *P. mirabilis*, *Salmonella* spp., *Shigella* spp.) are susceptible to amoxicillin.

A: Critical diameters are validated for *E. coli* only. For other Enterobacteriaceae, the activity of tigecycline is variable, the MIC should be determined.

B: Ignore the thin shoot in the zone of inhibition.

Extended Spectrum Beta-Lactamase producing strains

The phenotypic confirmation of strains producing extended-spectrum beta-lactamases (ESBL) was carried out according to the method described by Bakouret *al.*²³. It consisted of looking for the champagne cork or funnel images that appear between the discs of amoxicillin-clavulanic acid, cefepime, and ceftriaxone antibiotics on Mueller-Hinton agar.

Molecular characterization of virulence and antibiotic resistance genes by PCR

DNA extraction

Extraction of DNA of *E. coli* and *Klebsiella sp.* strains was carried out by thermal shock, which consists of boiling the microbial suspension in a thermomixer (Thermomixer Compact, Eppendorf) at 100 °C at 600 rpm for 10 min and then collecting 250 µl of the supernatant in 1.5 mL tubes after centrifugation at 12,000 rpm at 4° C for 10 min in a refrigerated centrifuge (HettichZentrifugen, Mikro 22 R).

PCR amplification of antibiotic resistance genes

PCR amplification of the resistance gene (*bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M}) was performed in a 50 µl reaction according to the values contained in Table n°2, the primers used are listed in Table n°3, and amplification conditions reported in Table n°4. The reaction mixture without DNA served as a negative control, and a strain containing the desired gene served as a positive control.

Table n°2: Reaction mixture for the amplification of resistance genes from *E. coli* and *Klebsiella sp.*

Reagents	Initial concentrations	Quantity (µL)	Final concentrations
PCR quality water	-	30.3	-
DNA sample			
PCR buffercolored	-	5	-
PCR buffer non colored	10X	5	1X
MgCl ₂	10X	5	1X
dNTP	25mM	3	1.5mM
Primer Forward	10mM	0.5	0.1mM
Primer Reverse	10µM	0.5	0.1µM
Taq polymérase	10µM	0.5	0.1µM
	5U/µL	0.2	0.02U/50µL
Total	-	50	-

Table n°3: Sequences of primers usedfor antibiotic genes resistance

Genes	Primers	Sequences of primers (5'-3')	Size (bp)	References
<i>bla</i> _{TEM}	TEM-F	5'-TTGGGTGCACGAGTGGGTTA-3'	465	[24]
	TEM-R	5'-TAATTGTTGCCGGGAAGCTA-3'		
<i>bla</i> _{SHV}	SHV-F	5'-AGGATTGACTGCCTTTTTG-3	392	[24]
	SHV-R	5'-ATTTGCTGATTTTCGCTCG -3'		
<i>bla</i> _{CTX-M}	CTXM1-F	5'-GGTTAAAAAATCACTGCGTC -3'	863	[25]
	CTXM1-R	5'-TTGGTGACGATTTTAGCCGC-3'		

Table n°4: Amplification or cycling conditions of resistance genes

Steps	Conditions (Temperature / duration)	
	<i>bla</i> _{TEM}	<i>bla</i> _{SHV/CTX-M}
Initial denaturation	94°C/5 min	94°C/5 min
Denaturation cycles	94°C/1 min	94°C/1 min
Hybridisation	50°C/1 min	60°C/1 min
Elongation cycles	72°C/1 min	72°C/1 min
Final extension	72°C/7 min	72°C/7 min
Number of denaturation cycles	30	30

PCR amplification of virulence genes of *E. coli*

Search of virulence genes (*eae*, *bfp*, *stx1* and *stx2*) amount *E. coli* strains were carried outby PCR amplification which were performed in a 50 µl (see table n°2). Primers used and amplification conditions are listed in Table n°5 and Table n°6 respectively.

Table n°5: Primer sequences used for *Escherichia coli* virulence genes

Pathogenic <i>E. coli</i>	Genes	Sequences of primers (5'-3')	Size (pb)	References
EPEC	<i>eaeA</i>	eae-F CACACGAATAAACTGACTAAAATG eae-R AAAAACGCTGACCCGCACCTAAAT	367	[26]
	<i>bfpA</i>	bfp-F TTCTTGGTGCTTGCGTGTCTTTT bfp-R TTTTGTTGTGTATCTTTGTAA	324	[26]
EHEC/ETEC	<i>stx1</i>	stx1-F GAAAGTCCGTGGGATTACG stx1-R AGCGATGCAGCTATTAATAA	130	[27]
	<i>stx2</i>	stx2-F ACCGTTTTTCAGATTTTACACATA stx2-R TACACAGGAGCAGTTTCAGACAGT	298	[27]

EPEC : Enteropathogenic *E. coli*
 EHEC : Entéro-hémorragic *E. coli*
 ETEC : Enterotoxigène *E. coli*
eaeA : *E. coli* Attaching and Effacing A
bfpA : Bundle-Forming-Pili A
stx1 : Shiga-toxin 1
stx2 : Shiga-toxin 2

Table n° 6: Amplification or cycling conditions of *Escherichia coli* virulence genes

Steps	Conditions (Temperature/duration)	
	<i>stx1 et stx2</i>	<i>Eae et bfp</i>
Initial denaturation	94°C/3 min	94°C/3 min
Denaturation cycles	94°C/30 s	94°C/30 s
Hybridisation	57°C/45 s	56°C/20 s
Elongation cycles	72°C/30 s	72°C/30 s
Final extension	72°C/5 min	72°C/5 min
Number of denaturation cycles		35

Migration and revelation of amplification products

The migration was done on 1.5% agarose gel with SyBr® Safe DNA gel (Invitrogen) (8 µl/100 ml) at 100 Volts for 25 mn and the visualization of the fragments obtained after migration was done with the Gel Doc EZ Imager BIO-RAD.

III. Results

E. coli and *Klebsiella sp.* distribution in streets hot beverages

According to **Table n°7**, *E. coli* and *Klebsiella sp.* strains were more isolated in tea at 5% and 2.5%, respectively.

Table 7: Distribution of *E. coli* and *Klebsiella sp.* strains in hot beverages

Hot beverages	Sample size (N)	<i>E. coli</i> n (%)	<i>Klebsiella sp.</i> n (%)	Total N (%)
Coffee	200	3 (1.5)	2 (1)	5 (2,5)
Tea	200	10 (5)	5 (2,5)	15 (7,5)
Milk	200	7 (3,5)	3 (1,5)	10 (5)
Total	600	20 (3,3)	10 (1,6)	30 (5)

Susceptibility of isolated *E. coli* strains to antibiotics

Several antibiotics from different families were tested, and the profiles of isolates were determined. The different diameters obtained were compared with the standards (EUCAST/CA-SFM) to determine whether the strains were resistant or sensitive to each of the antibiotics. It was found that *E. coli* strains were resistant to 100% of piperacillin, followed by amoxicillin (50%), and ticarcillin (45%). However, cefepime, amikacin, and gentamicin had the greatest effect on 100% of *E. coli* (**Figure 1**).

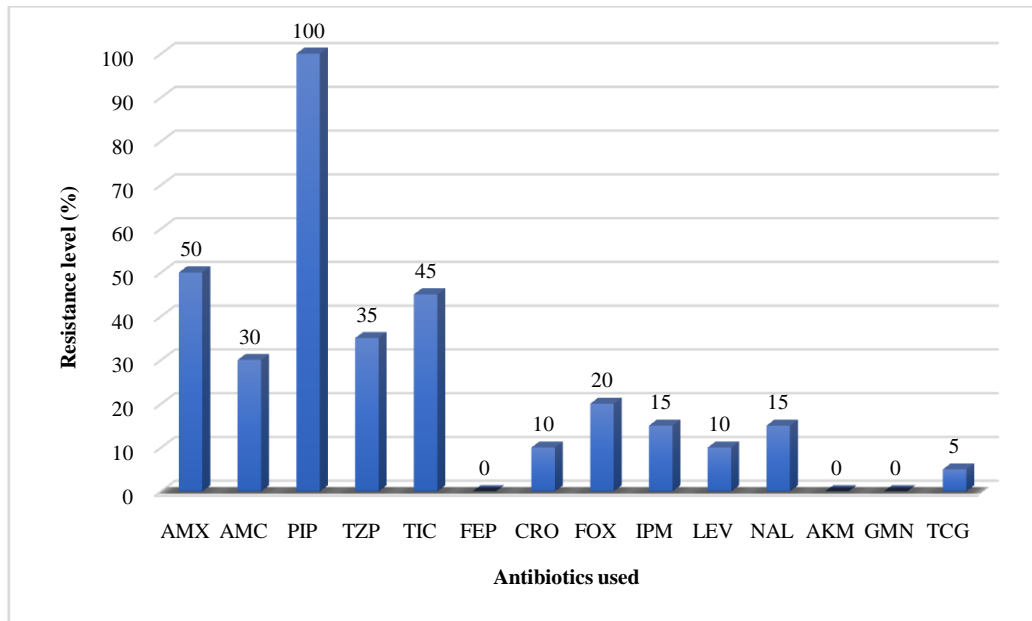


Figure 1: Antibiotic resistance of *E. coli* isolated from hot beverages

AMX: Amoxicillin; AMC: Amoxicillin-Clavulanic acid; PIP: Ticarcillin; TZP: Piperacillin/Tazobactam; TIC: Ticarcillin; FEP: Cefepime; CRO: Ceftriaxone; FOX: Cefoxitin; IPM: Imipenem; LEV: Levofloxacin; NAL: Nalidixic acid, AKM: Amikacin; GMN: Gentamicin; TCG: Tigecycline

Susceptibility of *Klebsiella* strains to antibiotics

In **Figure 2**, the results of the susceptibility test showed that the *Klebsiella* strains are resistant to the majority of the antibiotics tested. They were all resistant to piperacillin (100%), followed by amoxicillin-clavulanic acid (90%), amoxicillin (80%), and tazobactam (70%). It should be noted, however, that the antibiotics tested on *Klebsiella* strains have a narrow range of susceptibility: the strains are susceptible to imipenem (20%) and amikacin (10%).

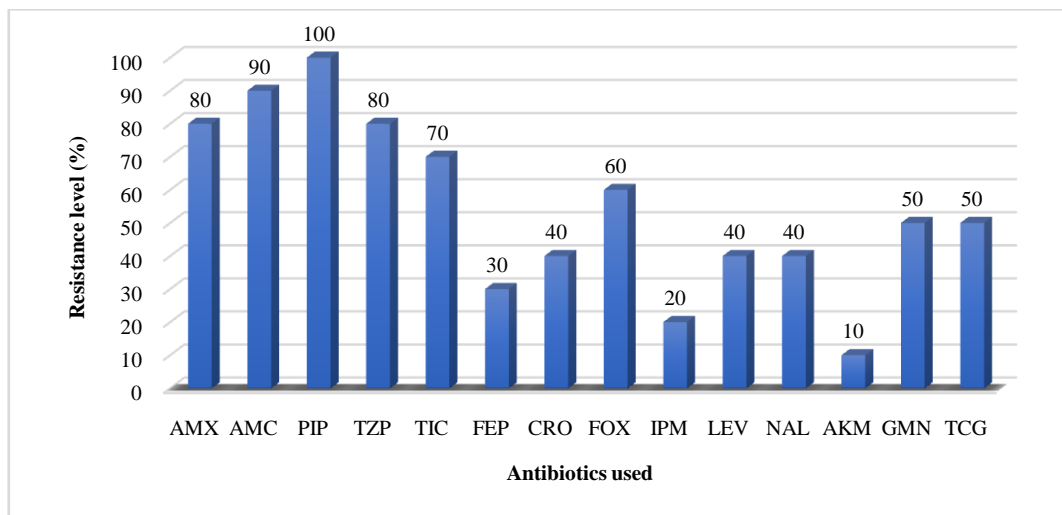


Figure2: Antibiotic resistance of *Klebsiella* isolated from hot beverages

AMX: Amoxicillin; AMC: Amoxicillin-Clavulanic acid; PIP: Ticarcillin; TZP: Piperacillin/Tazobactam; TIC: Ticarcillin; FEP: Cefepime; CRO: Ceftriaxone; FOX: Cefoxitin; IPM: Imipenem; LEV: Levofloxacin; NAL: Nalidixic acid, AKM: Amikacin; GMN: Gentamicin; TCG: Tigecycline

Prevalence of *E. coli* and *Klebsiella* with ESBL and antibiotic resistance genes

For 30 strains tested, 20 *Escherichia coli* and 10 *Klebsiella sp.*, 6.67% (2) of the *Klebsiella* strains produced extended-spectrum beta-lactamases (**Figure 3**). Furthermore, all strains of *Klebsiella sp.* producing extended-

spectrum beta-lactamases and one non-producing strain of extended-spectrum beta-lactamases possessed the beta-lactam resistance genes *bla_{TEM}* up to 30% (3) and 20% (2) to the *bla_{SHV}* genes, but none of the strains harbored the *bla_{CTX-M}* gene (Figure 4).

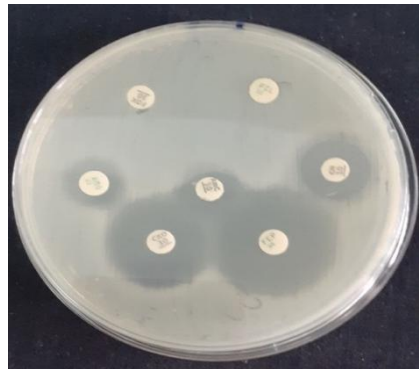


Figure 3 : ESBL production by *Klebsiella*

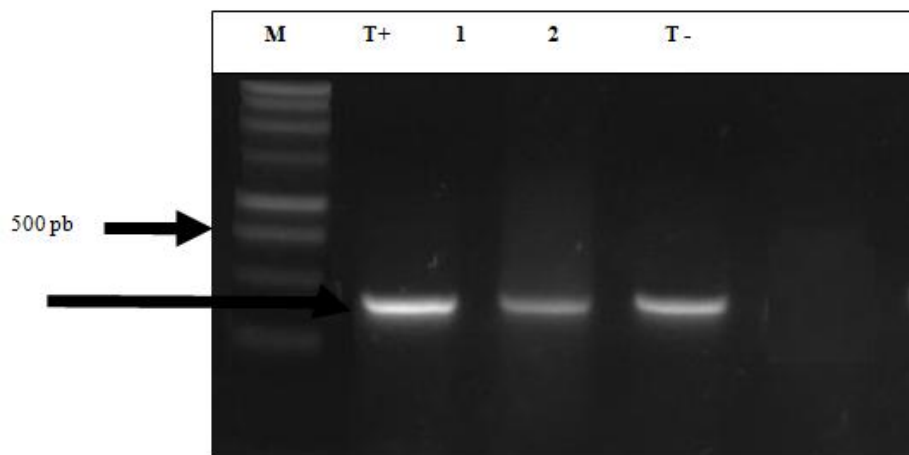


Figure 4 :Electrophoretic profile of *Klebsiella bla_{SHV}* gene

Column M: molecular weight marker,
 T+ column: positive control strain
 T- column: negative control
 Column 1, 2: strains analyzed harboring the *bla_{SHV}* gene

Pathogenic *E. coli* prevalence

Electrophoretic analysis of the four virulence genes (*eaeA*, *bfpA*, *stx1* and *stx2*) showed that only the *stx1* gene was present. It was detected with a prevalence of 5% (1/20). It could be STEC (Figure 5).

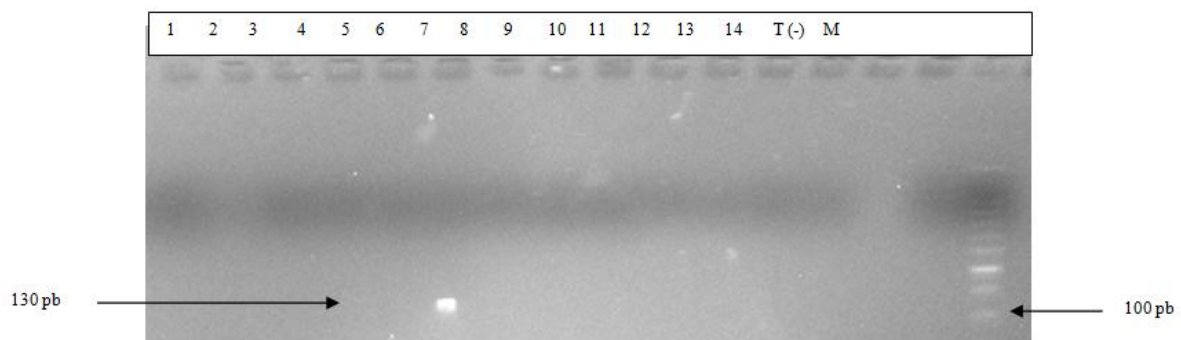


Figure 5: Electrophoretic profile of *E. coli stx1* gene

Column M: molecular weight marker,
T- column:negative control
Column 1, 2,3,4,5,6,7,8,9,10, 11,12,13:strainsanalysed

IV. Discussion

The objective of this work was to assess the health impact of street hot drinks due to the presence of *Escherichia coli* and *Klebsiella* spp. possessing virulence and antibiotic resistance genes. Street food offers a highly convenient source of low-cost food for the public^{3,10}. However, this commodity also contributes to the risks of foodborne diseases if food safety and hygiene measures are not considered²⁸. In this study, the presence of enterobacteria was assessed in three different types of hot beverages, such as coffee, tea, and milk, sold by street vendors. Enterobacteria is a suitable indicator to detect faecal contamination in food products^{29,30}.

On a set of 76 presumptive Enterobacteria isolates from previous studies by Atobla et al⁸, the distribution rate is higher in tea (5%) and lower in coffee (1.5%) for strains of *Escherichia coli*. Also, 50% of *E. coli* and *Klebsiella* strains come from tea and its ingredients. The presence of these microorganisms in these hot drinks can be explained in several ways. Indeed, several studies have shown that improper handling, poor hygienic practices, and lack of environmental control affect the safety of street-vended beverages^{3,10,11,31,32}.

Another fact that can explain this heat resistance is the presence of lipo-polysaccharides (LPS). Heat resistance is provided by LPS. Indeed, enterobacteria have O antigens or somatic antigens, which are wall antigens made up of lipo-polysaccharides and which are thermostable³³. Thus, insufficient and short-term heating cannot eliminate them. These contaminants can also occur after heating by microorganisms of the air during the mixing-stirring movement. Also, the misuse of disposable glasses, mint leaves, and other ingredients could be sources of contamination. In fact, the studies carried out by Atobla et al.⁸ noted that 5.6% of consumers of these drinks reported that they had negative effects such as diarrhoea (1.2%), nausea from street beverage vendors with coffee mobile cart. The studies conducted by Somilyet al.³⁴ showed that in the case of contamination linked to street food, the most frequently isolated species were *Escherichia coli* and *Klebsiella* sp.

E. coli was also used as an indicator bacterium for faecal contamination in drinks, allowing researchers to identify food and beverage samples with unacceptable levels of faecal contamination³⁵. *E. coli* is considered to be a more specific indicator bacteria to detect faecal contamination compared to faecal coliform because the most common test for faecal coliform also detects heat-resistant non-coliform bacteria³⁶. The presence of *E. coli* can also indicate the presence of pathogenic *E. coli* in food products. In our study, the result of 3.3% of *E. coli* obtained is lower than those obtained by BsadjoTchambaet al.⁶ in Burkina Faso after analysis of "bissap" and "gnamakoudji" with respective results of 12.70% and 20.70%.

Even at low doses (10–100 cells), enterohemorrhagic *E. coli* (EHEC) O157:H7 contaminated food can cause severe illnesses in humans, such as bloody diarrhea or hemolytic uremic syndrome³⁷. In this study, the virulence genes *stx2*, *bfpA*, *eaeA* were not detected. However, only one strain of *E. coli* producing shiga-toxin 1 (*stx1*) was detected. The prevalence of STEC in our study is therefore 5%. This frequency is slightly higher than that determined in the work carried out by Dadieet al.²⁷, who revealed that the presence of *E. coli* possessing virulence factors in food products is 4%. The presence of virulent *E. coli* (4%) in the food products analyzed is of the same order (2.6%) as that found by Cohen³⁸ during the analysis of beef and sausage in Morocco. However, El-Sharef³⁹, determining the quality of ice cream produced in Tripoli in Libya, obtained a prevalence of 6.2% of virulent *E. coli*. These results are much lower than those of Rigobeloet al.⁴⁰, who detected a prevalence of 49.1%. The number of strains (1) producing shiga-toxin 1 (STEC) was also obtained in unpasteurized milk during the study carried out by Dadieet al.²⁷. This study therefore confirms that the results relating to STEC in our environment are quantitatively insignificant. However, given the particular severity of STEC infections with the possibility of the occurrence of fatal cases⁴¹, the qualitative aspect of this result should attract the attention of the authorities.

Klebsiella pneumoniae is known to be a pathogenic bacterium that contaminates food during the processing and packaging stages. Therefore, practicing good hygiene towards raw food materials and utensils is an important feature of food vendors. In addition, environmental sanitation, personal hygiene, and immunity are some of the decisive factors influencing bacterial infection⁴². The results of our studies showed a prevalence of 50% of *Klebsiella* sp. in the tea, which is the most contaminated. According to studies by Atoblaet al.⁸ tea is the most consumed beverage, with a rate of 38.4%. As a result, consumers are exposed to disease risks, especially since *Klebsiella* opportunistic pathogens cause several infections in humans, including urinary tract infections (UTI), pneumonia, septicemia, meningitis, rhinoscleroma, ozaena, sinusitis, otitis, enteritis, appendicitis, and cholecystitis⁴³. However, our results (50%) are lower than those found by Tri Yahya et al.², who found a percentage of 89–99% contamination of the food and beverages analyzed. However, the prevalence of 13.1% obtained in our study is lower than that of 25.8% obtained by Gassem⁴⁴ in Saudi Arabia after analysis of Sobia, a fermented drink.

Some *E. coli* species are known to belong to Jarlier group 1, composed of enterobacteria naturally sensitive to beta-lactams⁴⁵. This resistance to beta-lactams can be explained by the adaptation of some of these strains to the molecules concerned and the acquisition of resistance factors over time. The evolution of *E. coli* resistance in Côte d'Ivoire has already been mentioned by Dadiet *al.*⁴⁶ and Guessend *et al.*⁴⁷, and the phenomenon has been part of an international context for about twenty years. This resistance concerned the family of penicillins, carbapenems, and third generation cephalosporins, but no resistance to fourth generation cephalosporins (cefepime) was observed. In the strains of *Klebsiella sp.*, a high level of resistance to the majority of the antibiotics tested was observed. These strains were resistant to antibiotics of the penicillin group by the production of class A beta-lactamases, also inactivating first, second, and third generation cephalosporins and inhibited by clavulanic acid⁴⁸. Indeed, the resistance of *Klebsiella* to this family confirms their belonging to group 2 of Jarlier⁴⁵. However, the 90% resistance to amoxicillin-clavulanic acid is due to their acquired resistance. As for resistance to the aminoglycosides tested, gentamicin and amikacin, *Klebsiella sp.* expressed low resistance to amikacin (10%) and to gentamicin (50%), while *E. coli* showed no resistance. These results are in agreement with the work of Ahouandjinouet *al.*⁴⁹, who revealed that the most active antibiotics against the microorganisms isolated in their study were gentamycin (98.56%), amikacin (87.05%) and imipenem (86.33%). These antibiotics are often the most effective for the treatment of enterobacteria infections, most often in combination with beta-lactams. However, their wide use has contributed to the emergence of resistant strains⁵⁰.

Results carried out according to the Antibiogram Committee of the French Society of Microbiology revealed that the majority of *E. coli* strains show variable sensitivity to the antibiotics tested. However, 100% of *E. coli* strains were resistant to piperacillin, 50% to amoxicillin, and 45% to ticarcillin. Among the ESBL-producing strains, the frequency of isolation of *Klebsiella sp.* was 6.67%, while that of *E. coli* was 0%. The frequency of *E. coli* isolation in these hot beverages is almost in agreement with the results of Ahouandjinouet *al.*⁴⁹, who noticed in their study that 97.12% of strains produced penicillinase against 0.7% of *E. coli* strains that produced ESBL. This rate is not far from the 0.4% observed by Mesa *et al.*⁵¹ in food. However, this result is very different from those reported in the hospital environment, which are 16% in Cameroon⁵² and 33.33% in America⁵³. Also, the studies carried out by Toudjiet *al.*⁵⁴ reveal in hospitals an isolation frequency of 51.13% of *E. coli* strains and 30.10% for *Klebsiella sp.* among the ESBL-producing strains. This difference can be explained by the fact that the strains studied in hospitals have been in constant contact with antibiotics and have therefore acquired certain resistances. Also, the frequency of ESBL-producing enterobacteria determined in this study is lower than that of previous studies, which reported a prevalence of 9% in 2008 and 56.2% in 2016 in ESBL-producing enterobacteria in Côte d'Ivoire^{47,55}.

The presence of genes coding for *bla*_{TEM} (30%) and *bla*_{SHV} (20%) confirms the resistance of strains of *Klebsiella sp.* to beta-lactams. The absence of a gene encoding *bla*_{CTX-M} in our study is in contradiction with studies performed by Livermore *et al.*⁵⁶, who in separate studies stated that the *CTX-M* gene was the most prevalent of the genes encoding *bla*_{CTX-M}. ESBL is found throughout the world and replaces the *TEM* and *SHV* genes in many countries. The absence of resistance genes in the strains of *E. coli* tested would confirm their sensitivity to the majority of antibiotics.

V. Conclusion

Hot beverages sold in the streets of Abidjan by vendors with coffee carts are indeed contaminated by species of *Escherichiacoli* and *Klebsiellasp.*. The sensitivity test carried out revealed more resistance to the antibiotics tested in the strains of *Klebsiellasp.*. All strains of *E. coli* and *Klebsiella* were resistant to piperacillin, and the majority were sensitive to amikacin and gentamicin. Molecular characterization of *E. coli* strains revealed the presence of one virulence gene and no resistance gene. On the other hand, in strains of *Klebsiella sp.*, *bla*_{TEM} and *bla*_{SHV} type resistance genes were detected. The strains of *E. coli* found in these beverages are therefore potentially capable of causing poisoning given the ability of one of the strains to produce toxins. Although the prevalence obtained is low, the risk of infection with enterotoxigenic *E. coli* exists for consumers of these beverages.

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Atobla Koua, et al. “ Antibiotic Resistance and Virulence Genes of Escherichia coli and Klebsiella sp. Isolated from hot beverages sold on the streets of Abidjan, Côte d'Ivoire.” *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 17(2), (2023): pp 17-26.