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Evaluation of the microbiological quality and antibiotyping of strains of *Escherichia coli* strains isolated from street foods made from beef sold in Daloa (Côte d'Ivoire)

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ABSTRACT

The study made it possible to assess the microbiological quality of beef-based foods sold in Daloa, but also to perform an antibiogram on *Escherichia coli* strains isolated during labour. To carry out the research, thirty-five (35) samples were collected by purchase in five (05) districts of the city of Daloa and then transferred to the microbiology laboratory for analysis. A count followed by isolation of the strains was carried out. An antibiogram was carried on the strains of *E. coli* isolated from cooked beef. The results obtained revealed that the level of yeasts, molds and total flora with the samples comply with the microbiological criteria established by the European Community. As for total coliforms of Enterobacteriaceae, Staphylococci, Enterococci and *E. coli*, some samples taken from certain areas of the city did not meet the established criteria. Regarding resistance tests carried out on 10 strains of *E. coli* in the presence of 11 antibiotics usually used in human therapy, there was a multi-resistance of the strains to certain antibiotics. The presence of these nonconforming microorganisms is often the cause of certain food infections.

INTRODUCTION

The galloping demographic growth of major cities in the world in general and Africa, in particular, has developed the street food sector. Street food has particularly become a phenomenon on of modern societies due to its nutritional and socioeconomic importance (FAO, 2004; FAO/OMS, 2005). Indeed, it is a recurring phenomenon in Africa. The sale of food on the outskirts of the streets is an activity resulting from the informal sector and which is a significant source of employment in urban areas, especially for some people whose level of education is not high and who could not find another job (République de Côte d'Ivoire (RCI), 2002; Bottari et al., 2006). In addition, street food provides urban populations with ready-to-eat foods with popular tastes and at acceptable costs. In Côte d'Ivoire, and mainly in Daloa, street foods offer consumers the possibility of eating at a lower cost. The privileged places of sale and consumption are essentially the street. Lack of access to clean water and waste disposal can turn food into food poisoning (Galzy & Guiraud, 2003; FAO, 2004; Codex Alimentarius Commission, 2007). Furthermore, the

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microbiological quality of these foods is often not guaranteed. Indeed, epidemiological data in hospitals showed a prevalence of 19% of diarrheal diseases in the world. Bacterial and viral diarrhea were estimated between 20-70% of cases (FAO, 2001). The causes have been linked to malnutrition including eating out of the home. The agents of faecal peril are widespread in the environment, in particular in water contaminating food and thus carrying out a true entero-food-enteric cycle. Street foods, by their definition, are very close to these environments threatening their hygienic quality at any time (Bryan, et al., 1997; Barro et al., 2006). They become a source of several diseases with digestive tropism (King et al., 2000). In addition, cases of food poisoning caused by street foods have been reported in several countries around the world (Barro et al., 2002b). The importance of street foods and canteen foods requires monitoring their safety in order to reduce the illnesses they can cause. (Geay et al., 2002; Barro et al., 2002a). In Côte d'Ivoire, several cases of food poisoning due to the consumption of street food were reported in the local press, such as 34 children in a school in Bingerville.

There is not enough data in Côte d'Ivoire in general and in Daloa in particular on the consumption of street food and the various infections it could cause. The objective of the present work is to assess the microbiological quality of beef-based foods sold in Daloa, but also to perform an antibiogram on *Escherichia coli* strains isolated during the work.

MATERIAL AND METHODS

Sampling

A total of 35 samples of cooked beef was collected from the streets of five neighborhoods in Daloa town to perform this work. They were collected by purchase and then transported to the laboratory for analysis.

Microbiological analysis

The preparation of the initial suspension and the decimal dilutions were carried out according to the ISO 6887-1 standard. 90 ml of enrichment broth consisting of Buffered Peptone Water (EPT) (BioRad, Paris, France) previously prepared according to the manufacturer's instructions is added to sterile stomacher paper containing 10 g of cooked beef meat. The whole is ground in a stomacher. The suspension is left at laboratory temperature for 30 min for revivification of the microorganisms.

Enumeration of spoilage germs

One milliliter of each dilution obtained was introduced into the Petri dishes. A quantity of 15 ml of the previously prepared medium is poured into the Petri dish. The whole is well homogenized. The inoculated dishes are left on the bench to solidify the agar. The boxes thus solidified are incubated at 25°C/7days for the enumerations of yeasts and molds (NF/ISO 16212 : 2011), at 30°C/24 h for total coliforms (NF V08-050, NF V08-060 and NF EN ISO 4832, 2006a, b), at 30°C/72 H for aerobic mesophilic organisms (NF V08-051), at 37°C/48 H for enterococci (ISO 7899-2, 2000) and at 37°C/24 h for *Enterobacteriaceae* (ISO NF ISO 21528-2, 2004; NF V08-054).

Search for potentially pathogenic germs

A quantity of 0.1 ml of each decimal dilution concerned is placed in a Petri dish containing 15 ml of agar previously prepared and poured. Then the 0.1 ml is spread on the surface of the agar using a sterile spreader. The inoculated dishes are incubated at 45°C/24 h for the detection and enumeration of *E. coli* (NF ISO 16649-2) and at 37°C for 24 to 48 h for the detection of *Staphylococcus aureus* (ISO 6888-1 and NF V08-057-1).

Antibiotyping of *E. coli* isolates

Ten isolates of *E. coli* were selected for performing the antibiogram. Its isolates were purified on nutrient agar and then confirmed by the API 20 E gallery. The antibiogram was carried out taking into account the work of Guesseud et al. (2012). The discs used were selected according to the recommendations of the Antibiotic Committee of the French Society of Microbiology (CA-SFM, 2019) (Table 1).

RESULTS AND DISCUSSION

Spoilage microorganisms

The majority of the samples analyzed have average loads which vary according to the districts and also according to the parameters sought. Thus, at the level of fungal flora, the highest load is 2727.27 ± 559.21 cfu/g from the Lobia quarter and the lowest is 2.73 ± 0.23 cfu/g and comes from the Sun quarter. At the level of the total flora, the highest load is 4738.52 ± 75.40 cfu/g (Tazibouo) and the lowest is 9.19 ± 2.31 cfu/g (Sun). Likewise for other spoilage flora such as enterobacteria, enterococci, and fecal coliforms, the highest loads are respectively 8089.31 ± 395.25 cfu/g (Tazibouo), 261.19 ± 50.08 cfu/g (Commerce), 7431.86 ± 94.05 cfu/g (Lobia) and the lowest loads all come from the sunny quarter and are respectively 13.80 ± 0.34, 14.48 ± 7.73, 49.93 ± 24.65 cfu/g (Table 2).

Table 1. Antibiotic discs used and their respective loads.

N°	Antibiotic	disc load (µg)	inhibition diameters (mm)	
			Target	Acceptables limits
1	Gentamicin (Gmi)	10	22-23	19-26
2	Ampiciln (Amp)	10	18-19	18-22
3	Ticarcilin (Tic)	75	27	24-30
4	Chloramphénicol (Chl)	30	24	21-27
5	Tobramycin (Tmn)	10	22	18-26
6	Imipenène (Ipn)	10	29	26-32
7	Ciprofloxacin (Cip)	5	33	29-37
8	Nalixidic acid (Nal)	30	25	22-28
9	Lovofloxacin (Lvx)	5	33	29-37
10	Céfépime (Fep)	30	34	31-37
11	Triméthoprim sulfaméthoxazole (Sxt)	1.25/23.75	26	23-29

Table 2. Average load per quarter and per desired germ.

Parameters	Global average load in cfu / g / quarter				
	Soleil	Labia	Commerce	Tazibouo	Lobia
Fungal flora	2.73±0.23	18.29±8.58	153.39±33.13	465.11±136.71	2727.27±559.21
Total flora	9.19±2.31	95.45±48.58	879.65±117.58	4738.52±75.40	2408.86±352.12
Entéro	13.80±0.34	192.61±6.26	3914.81±826.81	8089.31±395.25	1704.63±72.99
Enterococci	14.48±7.73	180.1±83.25	261.19±50.08	172.82±99.55	178.66±36.66
Coliforms F	49.93±24.65	346.61±150.06	1261.35±613.96	2171.91±106.56	7431.86±94.05

Entero, Enterobacteriaceae; **F,** fecal.

Table 3. Average load by quarter and by germs sought.

Parameters	Global average load in cfu / g / quarter				
	Soleil	Labia	Commerce	Tazibouo	Lobia
E. Coli	0.63±0.09	46.49±59.34	33.44±11.86	42.79±12.21	43.76±14.25
S. aureus	106.81±46.5	165.16±13.73	1057.16±148.83	267.20±191.06	952.26±76.19

Potentially pathogenic microorganisms

The majority of the samples analyzed have overall average loads which vary according to the districts and also according to the parameters sought. In addition, for potentially pathogenic species such as *E. coli* and *S. aureus*, the highest loads are respectively 46.49 ± 59.34 cfu/g (Labia), 952.26 ± 76.19 cfu/g (Lobia) and the lowest loads were obtained respectively at the Sun district with 0.63 ± 0.09 cfu/g and 106.81 ± 46.5 cfu/g (Table 3).

Resistance rate of *E. coli* strains to antibiotics

Strains of *E. coli* isolated from cooked beef sold in quarters show varying resistance to the antibiotic molecules tested. The highest resistance rate is observed at the level of ticarcillin with 80% while the lowest rate of resistance is at 10% observed at the level of ciprofloxacin. In addition, two strains were resistant to both gentamicin and chloramphenicol with 20% each, three strains to nalidixic acid and to tobramycin with a respective rate of 30% each. No resistance was observed with the imipenene molecule and that of levofloxaxin (Table 4).

Strain resistance to beta-lactams

In this study, beta-lactams are represented by the molecules of ticarcilin, ampicillin, imipenene and cefepime. The rates of resistance to beta-lactams were very variable. Ticarcilin has a resistance rate of 80% while ampicilin and

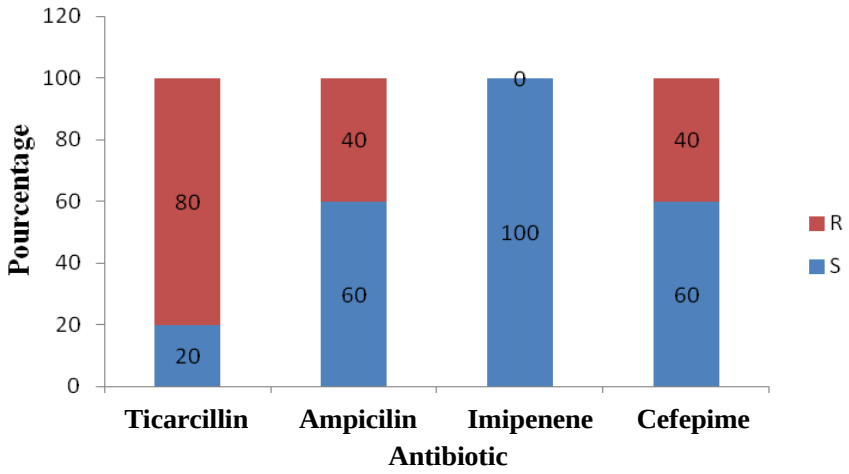

Figure 1. Sensitivity of *Escherichia coli* strains to beta-lactams.

Table 4. Resistance of the isolates of *E. coli* to antibiotics.

Antibiotics discs	Resistant strains	Percentage (%)
Imipenem	0	0
Lovofloxacin	0	0
Ciprofloxacin	1	10.00
Gentamicin	2	20.00
Chloramphenicol	2	20.00
Nalidixic acid	3	30.00
Tobramycin	3	30.00
Ampicillin	4	40.00
Cefepime	4	40.00
Trimethoprim sulfamethoxazole	5	50.00
Ticarcillin	8	80.00

cefepime have a resistance rate of 40% each. No strain resistance has been demonstrated for imipenem. The rates of resistance to antibiotics of the isolated strains are shown in Figure 1.

Resistance of isolates to other antibiotics

The other antibiotics have varying resistance ranging from 10% for Ciprofloxacin, 20% for gentamicin and chloramphenicol, 30% for tobramycin and nalixidic acid, 50% for trimethoprim sulfamethoxazole. Furthermore, no resistance strain has been demonstrated for levofloxacin (Figure 2).

Multi-resistant strains

The majority of the strains tested 90% or (9/10) were considered to be multi-resistant strains. Thus, 20% of the strains tested are resistant to two and three antibiotics, 40% to four antibiotics and 10% of the strains to five antibiotics. The distribution of these strains is mentioned in Figure 3.

DISCUSSION

The count of germs made it possible to highlight the spoilage flora and potentially pathogenic species in this study. Regarding yeasts and molds plus the total flora, all the samples analyzed during the work comply with the defined microbiological criteria. But with regard to total coliforms, enterobacteriaceae, enterococci, staphylococci and *E. coli* there is a non-compliance of some samples collected in some neighborhoods of the city of Daloa. In fact, this contamination can be due to the poor hygienic quality of the meat following poor production and transport conditions, but also on the distribution channels. Also, it

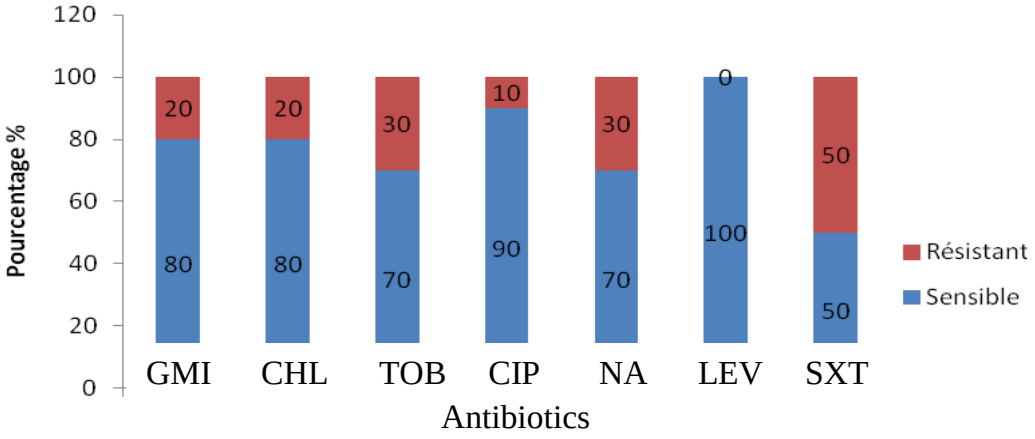

Figure 2. Sensitivity of *Escherichia coli* strains to other antibiotics.
GMI, Gentamicin; **CHL,** chloramphenicol; **TOB,** tobramycin; **CIP;** ciprofloxacin; **NA,** nalixidic acid; **LEV,** levofloxacin; **SXT,** triméthoprim sulfamethoxazole.

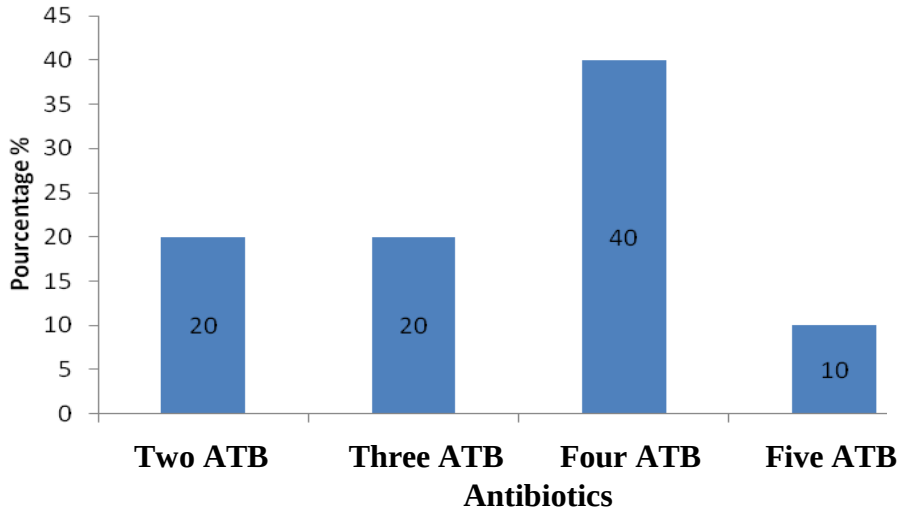

Figure 3. Distribution of the rates of multi-resistant strains.

could be contaminated during cooking by kitchen utensils, the many manipulations of vendors or the environment in which the trade takes place (Haeghebaert, 2002; FAO/OMS, 2005).

Furthermore, this microbial flora could undoubtedly represent the microorganisms that the food industry faces. Food is rarely sterile. They usually contain microorganisms that are mostly harmless, some of them are useful. A distinction is made between spoilage microorganisms which can be the cause of organoleptic or nutritional degradation and lead to a reduction in the shelf life of food.

Analysis of the spoilage flora of cooked beef sold in the streets of Daloa revealed the presence of many different microorganisms. Thus in this study, the analyzed samples were found to be contaminated by microorganisms with loads greater than the tolerable thresholds set by the European Community (2007).

These results corroborate with those of (Mughole, 2012) according to which 67% of sellers do not keep their food cold, 45% do not heat their food and finally 22% of sellers do not clean their hands before serving the food, which allows contamination of these foods and probably a proliferation of spoilage germs. Moreover, all the samples analyzed were positive for enterobacteria, enterococci, fecal coliforms, total flora and fungal flora. The results are similar to those reported by Ahouandjnou et al. (2015) who indicated a percentage of 100% incrimination of Enterobacteriaceae in the contamination of cooked bovine

meat in Benin.

However, these results do not agree with recent studies carried out by El Basett (2017) who highlighted a percentage of predominance of fecal coliforms of around 66.67% in cooked beef sold in the street. Similarly Lazar (2013) in a study found a predominance of faecal coliforms of more than 70% in cooked beef. Moreover Meftah and Souni (2017) in a recent study affirmed that fecal coliforms are microorganisms most incriminated in the contamination of cooked beef sold in the streets.

The study shows that cooked beef products were contaminated with Staphylococcus aureus. Humans are the main source of contamination of food with staphylococci presumed pathogens generally assimilated to *S. aureus*. It is the reservoir for several species of staphylococci that it harbors in the nasal passages and in the throat, especially in cases of colds. They are also present on wounds and burns, on the skin, hair, and in the ears. *S. aureus* is pathogenic due to its ability to secrete a heat-stable toxin that exerts both toxic and antigenic properties in the infected person.

The level of contamination of cooked beef sold in the street varies from neighborhood to neighborhood. Several previous studies by Cocolin et al. (2004) and Shale et al. (2005) on cooked beef have also indicated that the latter are important vehicles of *S. aureus*. Fang et al. (2003) in a previous study carried out in Taiwan revealed the presence of Staphylococcus at 88% in beef sandwiches against 100% in this study. This could be explained by poor hygiene of the sellers but also by a sales environment which is very unhealthy. Also, Shale et al. (2005) in one study indicated that it was during slaughter that *Staphylococcus* spp. contaminate beef, hence their presence in cooked beef sold in the street. In this study, the *E. coli* load in the majority of the sampled neighborhoods is beyond the standard established by the European community (2007). This presence is due to the lack of hygiene which affects the sale of many street foods such as meats which are sold in an unsanitary environment.

In this study, the resistance of *E. coli* strains isolated from cooked beef was assessed by performing an antibiogram. Thus, the isolates were tested on betalactamins such as ticarciline, ampicillin, imipenene and cefepime and also on other antibiotics such as aminoglycosides, phenicols, sulfonamides-trimethoprim and fluoquinononones. In addition, monitoring bacterial resistance to antibiotics, both in humans and animals, is important, with the aim of implementing a strategy to prevent the spread of multi-resistant bacteria (Adcock & Saint. 2001; Duijkeren et al., 2001; Delmas et al., 2006).

In order to set up a network to monitor bacterial resistance to antibiotics in men, this study was undertaken and allowed the testing of 10 strains of *E. coli* in the presence of 11 antibiotics usually used in human therapy.

The strains tested in our study showed a high level of resistance to certain antibiotics such as ampicillin, ticarcilin, and even newer, broad-spectrum antibiotics such as cefetime.

Teshager et al. (2000) also reported high resistance to ampicillin in Spain. This is the case reported by other authors in China who report 79% resistance to ticarcilin in 2004 (Yang et al., 2004). In the USA in 2012, authors reported 34.1% resistance to ampicillin (Daniel et al., 2012). Furthermore, no strain tested produced broad-spectrum beta lactamase, this was also reported in 2012 in the United States by Daniel et al. (2012). These resistance rates obtained are lower than those obtained by Émilie et al. (2008) in France, who demonstrated that 60% of the strains tested were resistant to amoxicillin. All strains were susceptible to imipenem; these results are similar to those of Seck (2005) in Senegal who showed a 100% rate of sensitivity for imipenem. Regarding other antibiotics, trimethoprim-sulfamethoxazole and chloramphenicol showed the highest rates of resistance. The high rate of resistance to trimethoprim-sulfamethoxazole (50%) is comparable to that obtained by Émilie et al. (2008), who showed a resistance rate of 64%. By the way, Seck (2005), in his work shows even higher rates of around 89%.

Ampicillin is a molecule of choice for the treatment of enteritis. The frequency of use of these molecules favored the selection of resistant strains. The high rates of resistance (40%) justify the need to determine the phenotype of resistance to beta-lactams. The presence of these strains could be considered a public health risk especially for consumers of street foods (Bottari et al., 2006; Bergeron et al., 2012). Since the 1950s, chloramphenicol has been the major compound of the phenicols family most widely used against numerous bacterial infections, both in human and veterinary medicine (Cohen, 2006; Kuo et al., 2009; Meunier et al., 2010).

Conclusion

Street eating is usually done in an unsanitary environment that can lead to foodborne illness. The study carried out on some street foods in the city of Daloa, revealed compliance of average loads with the microbiological criteria of total flora and yeasts and molds. What is not the case with total coliforms, Enterobacteriaceae, Enterococci, Staphylococci and *E. coli* where the average loads are beyond microbiological criteria. The antibiogram performed on 10 strains of *E. coli* in the presence of 11 antibiotics, revealed a multi-resistance of the latter.

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