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(RESEARCH ARTICLE)

RESISTANCE GENES FROM *E. coli* ISOLATED FROM BOVINE MEAT IN THE PORT-BOUËT MUNICIPALITY, ABIDJAN (CÔTE D'IVOIRE)

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ABSTRACT

One of the major consequences of the misuse of veterinary drugs in livestock farming is the emergence of strains resistant to the antibiotics used. *E. coli* is very often involved in cases of poisoning. This study aimed to determine the level of circulation of multidrug-resistant *Escherichia coli* associated with meat consumption in Côte d'Ivoire. Microbiological analysis of 150 beef samples (50 from slaughterhouses, 50 from markets, and 50 from braised meat) enabled us to isolate 181 strains of *Escherichia coli*. These strains were phenotypically and molecularly characterized respectively for their multidrug resistance capability and for the possibility of possessing antibiotic resistance genes: *blaSHV*, *blaIMP*, and *blaTEM*. As results, the *E. coli* contamination rate was 16% for braised meat and 22% for fresh meat from slaughterhouses and markets. The average load of *E. coli* in the matrices ranged from 2.2 ± 0.05 to 5.8 ± 0.1 logCFU/g. The prevalence of *E. coli* was 60.2%, 33.7%, and 06.1% for samples from markets, slaughterhouses, and braised meat, respectively. Of 41 multidrug-resistant *E. coli*, 29.3% and 17.1% were resistant to the *blaSHV* and *blaIMP* resistance genes, respectively. The emergence of antibiotic resistance highlights the need for training in the better use of veterinary drugs.

Keywords: Bovine meat, *E. coli* resistant, Resistance genes, Port-Bouët, Abidjan

1 INTRODUCTION

Ensuring the quality, safety and wholesomeness of food is an important issue in achieving the Global Development Goals (SDGs) [1]. Indeed, many populations are faced with a significant number of cases of foodborne diseases, particularly those resulting from the consumption of contaminated animal products [1, 2].

Meat can be prepared and eaten in a number of ways, including as grilled, braised, or boiled. It is a necessary human diet [3]. The braised form, commonly known as “Barbecue” in Europe, “Dibi” in Senegal, “Tchatchanga” in Benin and “Choukouya” in Côte d'Ivoire, is extremely popular with the Ivorian population. It is frequently found in places of relaxation and leisure, and at festive events [4, 5]. However, despite the high nutritional value of beef, it remains a foodstuff that is highly sensitive to spoilage. Indeed, the various stages from slaughtering to cutting, from carcass distribution to storage, as well as the conditions under which beef is sold, can lead to contamination, particularly by bacteria such as *Escherichia coli* [4, 6].

Animal products' food safety insecurity is generally caused by a number of variables, such as the rise in different virulence factors and the development of microorganism resistance to antimicrobial drugs, which results in frequent and challenging-to-control illnesses.

This topic can be further explored by looking more specifically at antibiotic resistance through virulence genes, leading to increased therapeutic failures and trans-ecosystemic dissemination of antibiotic resistance risk genes; resistance to antiseptics and disinfectants [7]. Furthermore, it has been reported that antimicrobial resistance also finds an explanation in biofilm formation.[8, 9] In fact, biofilms are the main form of bacterial development in natural environments and especially in agri-food chains [8, 10]. These are weathering or pathogenic flora, attaching or adhering to solid surfaces and, where appropriate, actively multiplying to form colonies [9]. Biofilms have been found on the surface of foodstuffs such as meats, cheeses and fresh vegetables, as well as in the industrial environment [11]. The consequences of this emergence are of greater concern for the most vulnerable population groups, such as children, pregnant women, the elderly and immunocompromised individuals [12, 13].

In Côte d'Ivoire, several studies have been carried out on the characterization of *Escherichia coli* isolated from milk, beef, infant foods and fourth range products [3, 14]. However, few studies have been carried out on antibiotic resistance, the presence of virulence in *E. coli* strains isolated from bovine-derived matrices.

The overall aim of this study was to determine the level of circulation of multiantibiotic-resistant *Escherichia coli*.

2 MATERIALS AND METHODS

The biological material used included fresh beef (*Bos taurus*) and charcoal-grilled beef “Choukouya” from the slaughterhouse and market in the municipality of Port-Bouët (**Figure 1**).



Fresh meat from the slaughterhouse



Braised beef



Butcher handling fresh meat at the market

Figure 1: Different bovine matrices used

2.1 Samples collection

For the study, 500 g samples of fresh beef were collected from the slaughterhouse and market in the municipality of Port-Bouët from butchers. 100 g samples of braised “Choukouya” meat were collected from braised meat vendors. The

vendors were selected at random. In total, one hundred and fifty (150) samples were taken, including fifty (50) samples of each type of beef over a period of ten (10) weeks (**Table 1**).

The selection of 50 samples per group (slaughterhouse, market, choukouya) ensures a rigorous balance between the cohorts. This standardized sample size (n = 50) is statistically sufficient for applying mean comparison tests or non-parametric tests. Sampling over 10 weeks (i.e., 5 samples per meat type per week) allows for the consideration of occasional environmental variations. This approach provides an accurate picture of the overall meat quality over the study period. The sample size also takes into account scientific rigor and the cost of the analyses.

As inclusion criteria, the fresh meat (slaughterhouse and market) comes exclusively from bovine carcasses inspected and validated by the veterinary services of the Port-Bouët slaughterhouse, and is neither frozen nor processed. Sampling was carried out with the agreement of the butchers and retailers. For the beef choukouya (braised meat), the meat was cut, seasoned, and braised according to the traditional preparation method. The ready-to-eat choukouya were collected directly from vendors with permanent or semi-permanent establishments in the Port-Bouët district. They were selected randomly.

Table 1: Size distribution and different matrices used and sampling location.

Sampling sites	Matrices used	Sample size
Port-Bouët slaughterhouse	Slaughterhouse fresh meat	50
	Braised beef	50
Port-Bouët market	Fresh market meat	50
Total		**150**

2.2 Determination of Escherichia coli load

The first step was to prepare the mother suspension and decimal dilutions down to 10^{-2} for braised meat (choukouya) and 10^{-6} for fresh beef. *E. coli* strains were then counted using the surface enumeration technique on pre-cast TBX medium (Biokar, Paris, France) as described in ISO 16649-2. An inoculum of 0.1 mL of each decimal dilution was transferred using a sterile pipette, onto the surface of pre-cast TBX medium. The inoculum was then carefully spread using a sterile spreader. After drying, plates were sealed at room temperature and incubated in an oven (MEMMERT, Germany) at 44°C for 24 to 48 hours. Blue colonies were counted, and plates with colonies between 15 and 150 were selected.

2.3 Phenotypic and biochemical identification of strains

Characteristic *E. coli* colonies were replicated on ordinary agar and incubated at 37°C for 24 hours. Gram staining tests, the oxidase test and inoculation with Le Minor's reduced rack elements (urea-Indole medium, Kligler-Hajna medium, Lysine-iron medium, mannitol-mobility-nitrate medium and Simmons citrate medium) were performed on presumptive *Escherichia coli* colonies.

2.4 Antibiotic susceptibility profile of the isolated E. coli strains

To determine the phenotypic resistance of the strains, an antibiogram was performed according to the recommendations of the Antibiogram Committee of the French Society for Microbiology (EUCAST/CA-SFM) [15].

The bacterial inoculum was prepared by suspending 1 or 2 isolated colonies (16-24 hours) in 5mL of 0.85% NaCl solution. The mixture was vortexed until homogeneous, and the suspension was calibrated to a 0.5 McFarland standard.

Mueller-Hinton agar plates were inoculated by swabbing. A sterile swab was dipped into the bacterial suspension and pressed against the inside wall of the tube to remove excess liquid. The entire agar surface was then streaked very closely in three successive passes to ensure complete coverage.

Antibiotic discs from various families (Bio-Rad, Marnes, France) were applied to the surface of the inoculated agar (Table 2). The discs were placed using a manual dispenser or sterile forceps, applying light pressure to ensure full contact without sliding.

A minimum distance of 15 mm was maintained between outer discs and the edge of the plate, with at least 30 mm between adjacent discs to prevent overlapping inhibition zones. The Petri dishes were then incubated upside down at 37°C under aerobic conditions for 24 hours.

Using a caliper, the diameter of the inhibition zone around each antibiotic disc was measured. Results were interpreted as Susceptible (S) or Resistant (R) based on EUCAST/CA-SFM [15] criteria.

Table 2: Antibiotic discs and their concentration for *E. coli* antibiogram

FAMILY	Antibiotics	Abréviations	Concentrations (µg)
PENICILLINS	Ticarcillin + clavulanic acid	TCC	75
	Ticarcillin	TIC	75
	Piperacillin	PIR	30
CEPHALOSPORINS	Cefepime	FEP	30
	Ceftazidime	CAZ	10
CARBAPENEMS	Imipenem	IMP	10
QUINOLONES	Ciprofloxacin	CIP	5
MONOBACTAMS	Aztreonam	ATM	30
FOSFOMYCIN	Fosfomycine	FOS	10
POLYMYXINS	Colistin	CST	Nd

Ticarcilline-Clavulanic acid (TCC), Ticarcillin (TIC), Piperacillin (PIR), Cefepime (FEP), Ceftazidime (CAZ), Imipenem (IMP), Ciprofloxacin (CIP), Aztreonam (ATM), Fosfomycin (FOS), Colistin (CST), Nd (not determined).

3 MOLECULAR CHARACTERIZATION OF ESCHERICHIA COLI ISOLATES

3.1 Extraction and purification of E. *coli* DNA

Each of the presumptive *E. coli* isolates was revivified in 3 mL of Luria-Bertani (LB) broth at 37°C for 18 hours. This broth was inoculated onto Trypticase Soy or Tryptic Soy (TS) nutrient agar (Becton-Dickinson, MD 21 152, USA), poured and dried beforehand, then incubated at 37°C for 24 hours. A well-isolated colony was purified by streak plating on Luria-Bertani (LB) agar and incubated at 37°C for 18-24 hours. *E. coli* genomic DNA was extracted by thermal lysis and purified using the method described by Guardia *et al* [16]. Indeed, three (3) well isolated identical colonies of each strain were picked and suspended in a 1mL volume of sterile Milli-Q water (milli-Q™, Millipore Corporation, USA). After pulse vortex, a volume of 200 µL of each suspension was collected and placed in sterile, pre-labeled Eppendorf tubes. The tubes were first incubated at -20°C for 15 minutes and then at 95°C for 15 minutes to create thermal shock. After centrifugation (SIGMA) at 14,000 rpm for 10 min, the supernatant was collected in sterile Eppendorf tubes. DNA was purified with 500 µL of phenol-chloroform-alcohol-isoamyl (25:24:1) and centrifuged for 10 min at 13,000 rpm at +4°C. The upper aqueous phase was then collected in a new sterile Eppendorf tube, and 1/10th of a volume of 3M sodium acetate (ID: 3620574, USA) and 500 µL of absolute ethanol (Agilent Technologies, CA, USA), stored at -20°C, were added to the aqueous phase and incubated at -20°C for 18 hours. The next day, the tubes were centrifuged at 13,000 rpm for 20 min at +4°C and the supernatant removed. A volume of 1 mL of 70% ethanol stored at -20°C was added to the pellet. Tubes were centrifuged again at 13,000 rpm for 10 min at +4°C. The supernatant was then removed and the pellet dried in a dry water bath at 70°C and resuspended in 60 µL of sterile Milli-Q water (milli-Q™, Millipore Corporation, USA). Finally, the absorbance (260 nm and 280 nm) and concentration (ng/µL) of DNA in the extract were determined using a spectrophotometer (Eppendorf BioPhotometer plus, USA). The absorbance of genomic DNA was measured in the Ultraviolet (UV) light spectrum from 220 to 300 nm. DNA was diluted with sterile Milli-Q water to obtain a concentration close to 100 ng/µL. The diluted DNA was stored at -20°C and used as a template for polymerase chain reactions (PCR).

3.2 Preparation of the reaction mixture

The reaction mixture was prepared according to the method described by Nawaz et al. (2023) [17]. This 25 µL reaction mixture consisted of 16 µL of sterile Milli-Q water (milli-Q™, Millipore Corporation, USA), 5 µL of 5X concentration loading buffer, 1.5 µL of 2 mM MgCl2 (Promega Corporation, Madison, WI 53711-5399, USA), 0,2 µL of 10 mM dNTPs, 0.1 µL of each primer, 10 mM (Integral DNA Technologie, California, USA), 0.1 µL of Go Taq® G2 Flexi DNA polymerase final concentration 1.5U (Promega Corporation, Madison, WI 53711-5399, USA) and 2 µL of DNA template. Sterile Milli-Q water and reference strains EC ATCC 25922 were used as negative and positive controls respectively for each PCR reaction operation.

3.3 Identification (amplification) of resistance genes

Amplification of resistance genes (*shv, imp, tem*) was carried out according to the method described by Pournajaf et al. (2018) [18]. *Tem* and *shv* encode beta-lactamases (penicillin/cephalosporin resistance), and *imp* encodes a carbapenemase (critical carbapenem resistance). These are major markers of antibiotic resistance in *E. coli*. The amplification program included an initial 5 min denaturation at 95°C followed by a repeated cyclic phase. The cyclic phase was repeated 33 times with one amplification cycle comprising a 30-second denaturation step at 95°C, a 60-second primer attachment (hybridization) step at 65°C and a 90-second elongation step at 72°C. The amplification reaction ended with a final elongation of 5 min at 72°C. Samples were stored at +4°C until shutdown on the T9700 Thermocycler, AB (Applied Biosystems) made in China (Singapore). The amplification programs and nucleotide sequences of the primers used are described in **Table 3**.

Table 3: Primers used to determine resistance genes

	Primer Sequence (5'→3')	**Amplification program**	**Size**
Antibiotic resistance genes			
SHV	F : 5' TGGTTATGCGTTATATTCGCC-3' R : 5' GCTTAGCGTTGCCAGTGCT-3'	95°C, 5 min 33 x [95°C, 30 s; 65°C, 60s; 72°C, 90s] 72°C, 5 min;	867 pb
TEM	F: 5' ATAAAATTCTTGAAGAC -3' R: 5' TTACCAATGCTTAATCA-3'		1075 pb
IMP	F : 5'TGAGCAAGTTATCTGTATTC -3' R : 5' TTAGTTGCTTGGTTTTGATG-3'		740 pb

Abbreviations: *TEM*: TEMONEIRA-Patient Name; *SHV* : Sulfhydryl Variable; *IMP* : MβL Imipenemase

3.4 Electrophoresis of amplification products

For electrophoresis of amplification products, 1% gels were prepared in 1X TAE buffer containing 2.5mg/L ethidium bromide solution. A 5µL volume of a molecular weight marker (Bench Top, 1kb DNA Ladder, Promega Corporation, USA) introduced into the first well of the solidified gel was used as a reference scale.

Gene amplification products were revealed on the agarose gel after migration at 120 V for 30 min and visualized by illumination on a UV plate of an illumination device and photographed (Bio-Rad, Gel Doc EZ Imager, USA). The DNA band and its size were plotted against the positive control and PM (molecular weight) marker respectively.

3.5 Statistical analysis

Statistical analyses were performed using SPSS software (version 20.0). Depending on the nature of the data, the chi-square test, analysis of variance (ANOVA), and non-parametric tests were applied.

4 RESULTS

4.1 Proportion of E. coli contaminating matrices

Of the samples taken, 11 were contaminated with fresh meat from the abattoir, 11 with market meat and 8 with braised beef. The contamination rate of these matrices ranged from 16% to 22%. The results indicate that fresh meat from the abattoir and the market had an identical contamination rate (22%). However, the rate was lower for braised meat (16 %) (**Table 4**). The p-value of 0.6873 is well above the critical threshold of 5% (0.05). Although braised meat appears to have a slightly lower contamination rate than fresh meat (16 % vs. 22 %), the statistical analysis does not allow us to conclude that the sampling location influences the level of contamination. Even when directly comparing fresh meat (22/100) to braised meat (8/50), the difference remains non-significant (p = 0.5160).

Table 4: Meat contamination rates

Matrices used	**Fresh meat (n = 100)**		**Braised meat (n = 50)**
Sampling site	Slaughterhouse (n = 50)	Market (n = 50)	Slaughterhouse (n = 50)
Contamination	11	11	08
levels in beef	22	22	16

4.2 Average E. *coli* load of matrices

The average *E. coli* load of animal matrices ranged from 2.2 ± 0.05 to 5.8 ± 0.1 log CFU/g. Braised beef showed the lowest *E. coli* load at 2.2 ± 0.05 log CFU/g (**Table 5**), while the highest mean *E. coli* load was observed in fresh market meat samples (5.8 ± 0.1). Comparison of means demonstrates that all analyzed groups showed statistically significant differences ($p < 0.001$). The increase in bacterial load observed between samples from the slaughterhouse (3.6 ± 0.05 CFU/g) and those from the market (6.12 ± 0.10 CFU/g) confirms microbiological proliferation or cross-contamination during distribution. Finally, although heat treatment (braising) induces a drastic reduction in the bacterial population (2.20 ± 0.05 CFU/g), the residual load remains significantly different from a complete absence of bacteria.

Table 5: Average *E. coli* loads in matrices

Analyzed matrices	Load in log CFU/g of E. coli		
	Minimum	Average	Maximum
Fresh meat Slaughterhouse	3.41	3.6 ±0,05	4.91
Fresh meat Market	5.8	6.12±0,1	7.37
Braised meat of beef	2.1	2.2 ±0,05	2.67

Abbreviations: CFU: colony-forming units

4.3 Relative frequency (%) E. *coli* in matrices

The study showed a difference in the contamination of samples by *Escherichia coli* strains. The results show that the Relative frequency (%) of *E. coli* is 60.2% in market-fresh meat, 33.7% in abattoir-fresh meat and 06.1% in braised beef (**Table 6**). The p-value ($p < 0.0001$) is well below the critical threshold of 5%, and the null hypothesis of equivalence is rejected with high statistical significance. The type of matrix crucially influences the presence and survival of the strains.

Table 6: Relative frequency (%) of *E. coli* in matrices

Type of matrice analyzed	Number of samples	Number of strains	Relative frequency (%)
Market fresh meat	50	109	60.21
Slaughterhouse fresh meat	50	61	33.7
Braised meat	50	11	06.1
Total	150	181	100

4.4 Multidrug resistance genes harbour by our Escherichia coli strains

Figure 2 shows expanded resistance spectra. In fact, several *E. coli* strains isolated from fresh meat both from the abattoir and from the market showed more than 50% multidrug resistance.

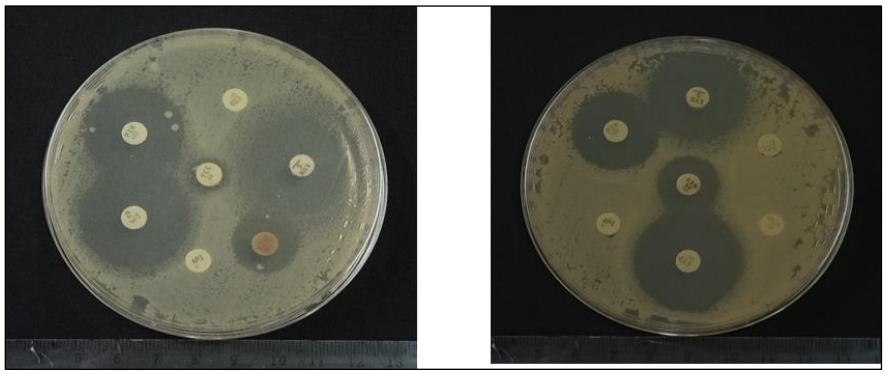


Figure 2: Phenotypic resistance of *E. coli* to antibiotics

4.5 Frequency of resistance genes in *E. coli* strains isolated from matrices

For a total of 181 *E. coli* strains isolated, 41 expressed multi resistance (22.65%). These multi-resistant strains (resistant to more than 3 antibiotic families) were screened for resistance carriers and potential biofilm-forming genes. **Figure 3**

shows the electrophoretic profile of resistance genes in the various strains. Overall, antibiotics resistance and biofilm-forming genes were more strongly detected in fresh meats. On the other hand, antibiotics resistance genes (*blaSHV* and *blaIMP*) were detected more in fresh market beef. The *blaTEM* gene was not detected in any of the matrices (**Table 7**). The frequency of the *blaSHV* gene detected was less than 30%, while that of the *blaIMP* gene was less than 20% (**Table 7**). The value of p ($p = 0.956 > 0.05$) indicates that there is no statistically significant difference in the relative proportion between the *blaSHV* and *blaIMP* resistance genes from one matrix to another.

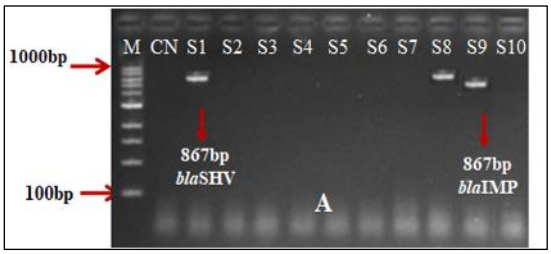


Figure 3: Electrophoretic profile of resistance gene amplification products

Gel A: Resistance genes: Line S1-S8-S9: Presence of resistance gene in E. coli.

Table 7: Frequency of resistance genes

Types of matrices	Percentage of resistance gene		
	bla_{SHV}	bla_{IMP}	bla_{TEM}
Market fresh meat	6 (14.6%)	4 (09.8%)	0 (00%)
Slaughterhouse fresh meat	4 (09.8%)	2 (04.9%)	0 (00%)
Braised meat	2 (04.9%)	1 (02.4%)	0 (00%)
Total	**12 (29.3%)**	**7 (17.1%)**	**0 (00%)**

5 DISCUSSION

E. coli contamination rates in the matrices studied ranged from 16% to 22%. The results indicate that fresh meat from the abattoir and from the market had identical contamination rates (22%). These contamination rates in fresh beef from the abattoir and the market are thought to be due to the lack of hygiene in the places where the animals are slaughtered and the meat sold. In addition, frequent unhygienic handling and cross-contamination have also been cited as reasons for contamination [19, 20].

These contamination rates and *E. coli* loads could also be explained by poor hygiene practices throughout the slaughtering, distribution and sale of beef. According to Ahouandjnou *et al.* [21] poor hygiene practices encountered in skinning and evisceration operations during the production of meat carcasses are recognized as risk factors in particular. Indeed, poor hygiene practices can lead to contamination of meat by a number of pathogens, including *Escherichia coli, Staphylococcus aureus, Clostridium spp*. In addition, the level of contamination and the *E. coli* load can be amplified by climatic conditions such as ambient temperature, water activity (aw) and poor product preservation [22].

In addition, the results indicated a lower *E. coli* count and load in braised beef. This could be justified by the fact that the cooking temperature not only eliminates microorganisms contaminating fresh meat, but also preserves their shelf life. This observation was made by Dibi et al. [23] who assessed the risk of *E. coli* contamination in braised beef. In contrast to this study, Dibi *et al*. [23] found higher *E. coli* loads in braised meat. Indeed, this difference in load could be explained by the study areas (Bouaké and Korhogo) characterized by the density of the population that frequents the market, the hygiene of the market, and the different and frequent handling undergone by this type of meat.

Thus, the prevalence of *E. coli* in fresh and braised beef could reflect the failure to observe hygiene rules and poor sanitary conditions observed at the point of sale [20, 24] and cross-contamination with soiled materials and packaging [25].

The results also showed that *E. coli* strains isolated from the matrices studied showed variable resistance depending on the antibiotics used. The level of antibiotic resistance in the *E. coli* isolated in this study could be linked to the overuse

of antibiotics during animal treatments. Indeed, the use of antibiotics in cattle would favor the emergence of multi-resistant strains of *E. coli*, which can be transmitted to humans through the consumption of contaminated meat or milk [26]. Furthermore, a study by Nasrollahian *et al*. [27] showed that resistant *E. coli* strains can serve as a reservoir of antibiotic resistance genes in the digestive tract, and that they readily exchange genetic material with other *E. coli* strains. In addition, *Escherichia coli* isolates from fresh beef tested for antibiotic resistance showed high resistance to certain antibiotics. This suggests that the isolated strains may have originated from the colonic contents disseminated by beef handlers during the butchering and post-slaughter cutting process.

Several studies on meat products have also reported strain resistance to beta-lactams [28, 29] and cephalosporins [29, 30]. These resistances may be due to the accessibility and overuse of antibiotics in veterinary and medical medicine.

However, the level of antibiotic resistance in *E. coli* from braised beef showed high sensitivity to all antibiotics. This increased sensitivity can be attributed to the proper cooking of meat at high temperatures, which can destroy the bacteria present, including those that might be resistant to antibiotics. If consumers cook meat properly, this can help maintain a low level of antibiotic resistance in the final product. The logical consequence of this resistance is the presence of antibiotic resistance genes.

The study also revealed the presence of resistance genes in *E. coli*. The *blaSHV* gene (29.3%) was the most commonly detected, followed by *blaIMP* (17.1%) in all 41 strains studied. These results could explain the phenotypic resistance to penicillins and cephalosporins observed [31]. Similar results were observed by Benie *et al*.[32], who reported in their study that the prevalence of these *blaSHV* genes can also be explained by the fact that most extended-spectrum beta-lactamase (ESBL) strains belong to the Sulfhydryl Variable (SHV) beta-lactamase families.

Resistance genes (*blaSHV* and *blaIMP*) were detected more in fresh than in braised beef. This may be because antibiotic-resistant and biofilm-forming bacteria may be more prevalent in the farmed animal environment due to the use of antibiotics in veterinary medicine and animal feed [33]. On the other hand, braised beef generally undergoes a cooking process that eliminates most of the microorganisms present, thus reducing the chances of detection of these genes [34]. The prevalence of *blaIMP* genes could be linked to carbapenem (imipenem) resistance in *E. coli* strains. Consequently, these enzymes could also indicate hydrolytic activity against carbapenems. However, the lack of epidemiological data on veterinary antibiotic use during this study means that the prevalence of *blaSHV* and *blaIMP* genes in commercially available fresh meat could also result from cross-contamination, environmental factors, post-slaughter hygiene issues, or horizontal gene transfer via mobile genetic elements during the distribution chain.

The complete absence of the *blaTEM* gene in all analyzed matrices is a notable finding. Indeed, this gene is one of the main determinants of beta-lactam resistance in Enterobacteriaceae. However, this genotypic absence does not guarantee the phenotypic susceptibility of the strains. Therefore, the observed beta-lactam resistance could be mediated by other detected enzyme families such as *blaSHV* or *blaIMP* (carbapenemase). However, the strict correlation between the presence of a gene and the expression of resistance in Petri dishes (phenotype) remains complex because a gene can be present but silent (not expressed), or vice versa. Phenotypic resistance can exist without these specific genes.

Following our study, we identified several limitations. Sampling was conducted exclusively in the Port-Bouët district of Abidjan; therefore, the results cannot be extrapolated to the entire country of Côte d'Ivoire. Furthermore, only three beta-lactam resistance genes (*blaSHV, blaIMP, blaTEM*) were targeted. The absence of the *blaTEM* gene in the strains, or the low frequencies observed for *blaSHV* and *blaIMP*, prevented us from covering the full genetic profile of the bacteria. Identification was limited to targeted PCR. This prevented the precise characterization of allelic variants, the mapping of mobile genetic elements (plasmids, integrons), and a detailed phylogenetic analysis. The lack of precise data on antibiotic use in livestock farming constitutes another limitation. It therefore remains difficult to definitively attribute the origin of resistance to the use of veterinary medicines in livestock farming rather than to environmental or human cross-contamination after slaughter, during distribution. These limitations make this research a local pilot study representative of Port-Bouët, but requiring larger-scale investigations to confirm the national dynamics of antibiotic resistance.

6 CONCLUSION

The safety of beef products is a very important criterion to be taken into account by the meat industry, which must offer the consumer every possible guarantee. The aim of this study was to characterize the health risk factors associated with *E. coli* in fresh and braised beef. The sensitivity of the molecular method using the 16S phylogenetic marker confirmed the identity of the *E. coli* strains isolated. The study showed the multi-resistance and potentiality of these strains to harbor resistance genes (*blaSHV*, *blaIMP*). It is important to take into account the presence of resistance genes and the ability to form biofilm when treating cattle prior to slaughter, to ensure maximum efficacy.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Author's Contribution

N'zebo Desiré KOUAME: Conceptualization, Validation, Methodology, Writing – **Moussa Konate**: original draft, Writing - review & editing. Visualization, Formal analysis. **Moumouny TRAORE:** Analysis and interpretation of data, - writing the article. **Benie Comoé Koffi Donatien:** Investigation, Formal analysis. **Atobla Koua:** Investigation, - proofreading and editing the manuscript; **Dadié Adjehi:** Formal analysis, - final approval of the version to be published.

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