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Microbiological quality and antimicrobial resistance of *Salmonella* spp. and *Escherichia coli* isolated from grilled meat

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ABSTRACT

In Chad, grilled meat has been well appreciated and consumed regularly. However, it could be the cause of many diseases if handled under unhygienic conditions. The objective of this study was to evaluate the microbiological quality and antimicrobial resistance profile of strains of *Salmonella* spp. and *Escherichia coli* isolated from grilled meat in the city of N'Djamena. Thirty producers were surveyed at least once from 02 August 2018 to 31 January 2019 and one grilled meat sample was collected from each of them. Total Mesophile Aerobic Flora (TMAF), Thermotolerant Coliform (TTC), *Escherichia coli* and *Salmonella* spp. counts, as well as susceptibility testing, were conducted at the Food and Nutrition Sciences Research Laboratory in N'Djamena according to standard methods. The level of the contamination of grilled meats was 83.33% for TMAF and 56.67% for TTC. The prevalence of *Escherichia coli* was 36.36%. Resistance was observed with some antibiotics especially those to the beta-lactam family. This study revealed that the grilled meat can expose the consumers to a sanitary risk. It should be stressed the strict application of good hygiene practices.

Keywords: grilled meat, hygienic, *Salmonella* spp., *Escherichia coli*, antimicrobial resistance, Chad.

INTRODUCTION

Meat has always been one of the bases of human nutrition. It is an important source of essential nutrients, including high quality protein, iron, zinc and B vitamins (Lecerf, 2014; Tidjani et al., 2013). However, because of its high water content and nutritional qualities, it is a favourable environment for bacterial growth (Dhraief et al., 2012). World meat consumption reached 323 million tons, and per capita consumption would average 41.8 kg worldwide and 31.5 kg in developing countries (FAO, 2017). In Chad, despite the rapid population growth and a large influx of

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refugees from Darfur, meat remains a staple food for Chadians. According to the Ministry of Livestock in Chad, the annual consumption was estimated at 22 kg/inhabitant between 2009-2016 (Livestock Ministry, 2008). The sector has made it possible to set up several grilling points in the cities and even in the most remote localities of the country. Grilled meat is regularly eaten by the entire population. But despite its socio-economic importance, the safety of grilled meat served in restaurants remains a major concern for consumers. They are handled under unhygienic conditions by the staff, thus promoting the contamination and multiplication of many microorganisms.

The contamination of these foods mainly by pathogenic germs would be a real health problem for the population (Douamba et al., 2022). According to the WHO, every year, 1 in 10 people become ill by consuming contaminated food and 420.000 die of it, including 125.000 children under five in the world (WHO, 2015). Of these food-borne diseases, diarrhoea ranks first with 230.000 deaths per year (WHO, 2015). In addition to pathogenic germs, some may be resistant to antibiotics, following their uncontrolled use in livestock and human health. This phenomenon has gradually contributed to resistance to different families of antibiotics (Muylaert et al., 2012). The selection of antibiotic-resistant pathogenic bacteria represents a danger to human health and a real public health problem (Varma et al., 2005).

In Chad, very little verifiable data exists on foodborne illness and antimicrobial resistance associated with ingestion of contaminated food of animal origin. Therefore, it is important to identify the pathogens involved, in order to implement specific measures to reduce the risk of contamination. In order to improve the hygienic quality of food sold on the street in Chad, this study aims to assess the hygienic quality of grilled meat served to consumers in the restaurants of the city of N'Djamena.

MATERIALS AND METHODS

Site, period and type of study

This is a prospective study that was conducted from 02 August 2018 to 31 January 2019 at the Research Laboratory for Food and Nutrition Sciences in N'Djamena, Chad. This study focused on the evaluation of compliance with good hygiene practices and the antimicrobial resistance profile of pathogens isolated from grilled meat in restaurants in the city of N'Djamena.

Survey

An investigation based on observations of palpable facts was carried out simultaneously at the time of sample collection in the 30 restaurants of the six boroughs of the city. The choice of these districts was based on the fact that the most popular restaurants of the city are in these districts. In addition, these establishments were located near markets, public roads and road guards.

Sampling

A total of 30 samples of grilled meat were randomly collected in sterile plastic bags labelled for five (5) restaurants in each borough. Thus, the samples were kept cold transported immediately to the laboratory for microbiological analyses.

Microbiological analyses

The microbiological analyses were carried out according to standard NF V 08-100:1998. Total aerobic microorganisms and thermotolerant coliforms were counted according to the standards (ISO 4833: 2003) and (ISO 4832: 2006) respectively. Briefly, the samples were ground in a sterilized mortar. 10 grams of each sample (grilled meat) was weighed and suspended in 90 mL of pre-sterilized buffered peptone water. The whole was homogenized with a Stomacher Lab blender. The supernatant was recovered in the flask and considered as the mother suspension. A subsequent dilution was performed (10^{-1} to 10^{-5}). After double-layer plating with 1 mL of different dilutions and an incubation period of 24 to 72 hours, all colonies were counted on two successive dilution plates containing at least 30 and at most 300 colonies for TMAF and 30 to 150 for coliforms. For the detection of *Salmonella* spp., 25 g of the grilled meat were

removed aseptically and homogenized in 225 mL of buffered peptone water. The search was carried out using the international standard method (ISO 6579: 2002). All analyses were performed under aseptic conditions. The results were expressed in terms of Colony Forming Unit (CFU/g) of sample and interpreted according to the criteria defined by Regulation (EC) 1441/2007 amending Regulation 2073.

Isolation and identification

After 24 to 48 hours of incubation, pink or bluish colonies with or without black centres isolated on *Salmonella-Shigella* agar from the pre-enriched Rappaport-Vassiliadis Soy broth solution were presumed *Salmonella* spp. Those with a bright metallic green reflection on the Eosin Bleu of Methylene agar were presumed *Escherichia coli*. These colonies were then sub cultured on Mueller-Hinton agar for different identification tests: Gram stain, oxidase test, and classical gallery. The API 20 E gallery was used to identify the *Salmonella* spp. *and E. coli* present in the samples. Strains were stored at -20°C in cryotubes containing Brain Heart Broth supplemented with 15% glycerol for subsequent analyses.

Antibiotic resistance profiles

Antibiotic was performed according to the Mueller-Hinton agar diffusion method. The diameters of the inhibition zones around each antibiotic were measured using millimetre graduated ruler. The interpretation of the results was made according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM, 2018). The choice of antibiotics was oriented according to their use in human and veterinary antibiotic therapy for the treatment of various pathologies in Chad. The antibiotics used according to the zones of inhibition in our study were presented in Table 1.

Data analysis

Data collected were analysed by Microsoft Excel software. The identification of the strains was realised by API Web software (version 4.1).

Table 1: Antibiotics used in the study.

Antibiotics	**Disk load (µg)**	**Inhibition zones (mm)**	
		Targets	**Acceptable limits**
Gentamicin (CN)	10	23	19-26
Amoxicillin Clavulanic acid (AUG)	30	21	18-24
Ceftriaxone (CRO)	30	32	29-35
Ciprofloxacin (CIP)	5	33	29-37
Norfloxacin (NOR)	10	32	29-35
Nalidixic acid (NA)	30	25	22-28
Cefotaxime (CEF)	5	28	25-31
Trimethoprim Sulfamethoxazole (SXT)	25	26	23-29
Imipenem (IMI)	10	29	26-32
Ampicillin (AMP)	10	18-19	15-24

RESULTS

Characterization of the producers and the meat supply chain

The different circuits from the raw material to the finished product sold in the restaurants namely: animal reception, bleeding, stripping, evisceration, post-mortem inspection, cooling, transportation, cutting, grilling and consumption are presented in Figure 1. The results of the survey conducted on the social characteristics of vendors in restaurants have been recorded in Table 2. It can be seen that the sellers of grilled meat in the restaurants were all men (100%) whose age varied between 25 and 40 years.

The majority of these vendors did not go to school (66.67%) and did not receive any specific training (90%). Nearly 73.33% of sellers were supplied with meat from central markets compared to only 26.67% of slaughterhouses. In these restaurants, 93.33% associated wood and Doum palm (*Hyphaene thebaica*) as a source of heat for grilling. In terms of water quality, 56.66% of vendors were supplied with water from the Chadian Water Company and 43.33% from borehole water.

Microbial contaminations

The main spoilage microorganisms (TMAF), hygiene indicators (TTC, TC) and pathogenic microorganisms (*Salmonella* spp. and *E. coli*) density in the samples are recorded in Table 3. The TMAF ranged between 1.11 x 10^6 and 1.85 x 10^7 CFU/g and the coliforms TC and TTC varied between 7.41 x 10^4 to 7.12 × 10^5 CFU/g and 1.30 × 10^3 to 9.37 × 10^4 CFU/g, respectively. *Salmonella* spp. was not isolated. The study notes that restaurants in districts A3, A5, A6 and A7 have much more dirty dishes than others.

The microbiological quality compliance of the grilled meat is summarized in the Figure 2. Only 3.33% of the samples presented an acceptable microbiological quality compliance (figure 2). Thermotolerant coliforms were identified with a compliance rate of 56.67%.

Antibiotic resistances of Escherichia coli isolates

The antibiotic resistances profiles of isolated *Escherichia coli* are presented in Table 4. Species of *Escherichia coli* isolated showed strong resistance to some antibiotics especially beta-lactams (ampicillin (100%), amoxicillin + Clavulanic acid (81.82%) and ceftriaxone (63.64%). However, low resistance was observed with trimethoprim-sulfamethazole and Nalidixic acid, that is 45.45% and 36.36% respectively.

Table 2: Social characteristics of the surveyed grilled meat vendors.

Characteristics of sellers	Proportion (%)	Characteristics of sellers	Proportion (%)
Sex and status		**Outfit**	
Men	100	Work clothes	76.67
Females	0	Regular dress	23.33
Married	86.66	**Used water**	
Single	13.34	Chadian Water Company	43.34
Age		Drilling	56.66
25-30	56.67	**Heat source**	
31-40	43.33	*Doum* palm (*Hyphaene thebaica*)	93.33
Level of education		Simple wood	6.67
Not in school	66.67	**General hygiene**	
Primary level	33.33	Staff	39.59
Place of supply of meat		Environment	31.67
Market	73.33	Equipment	28.74
Slaughterhouse	26.67		

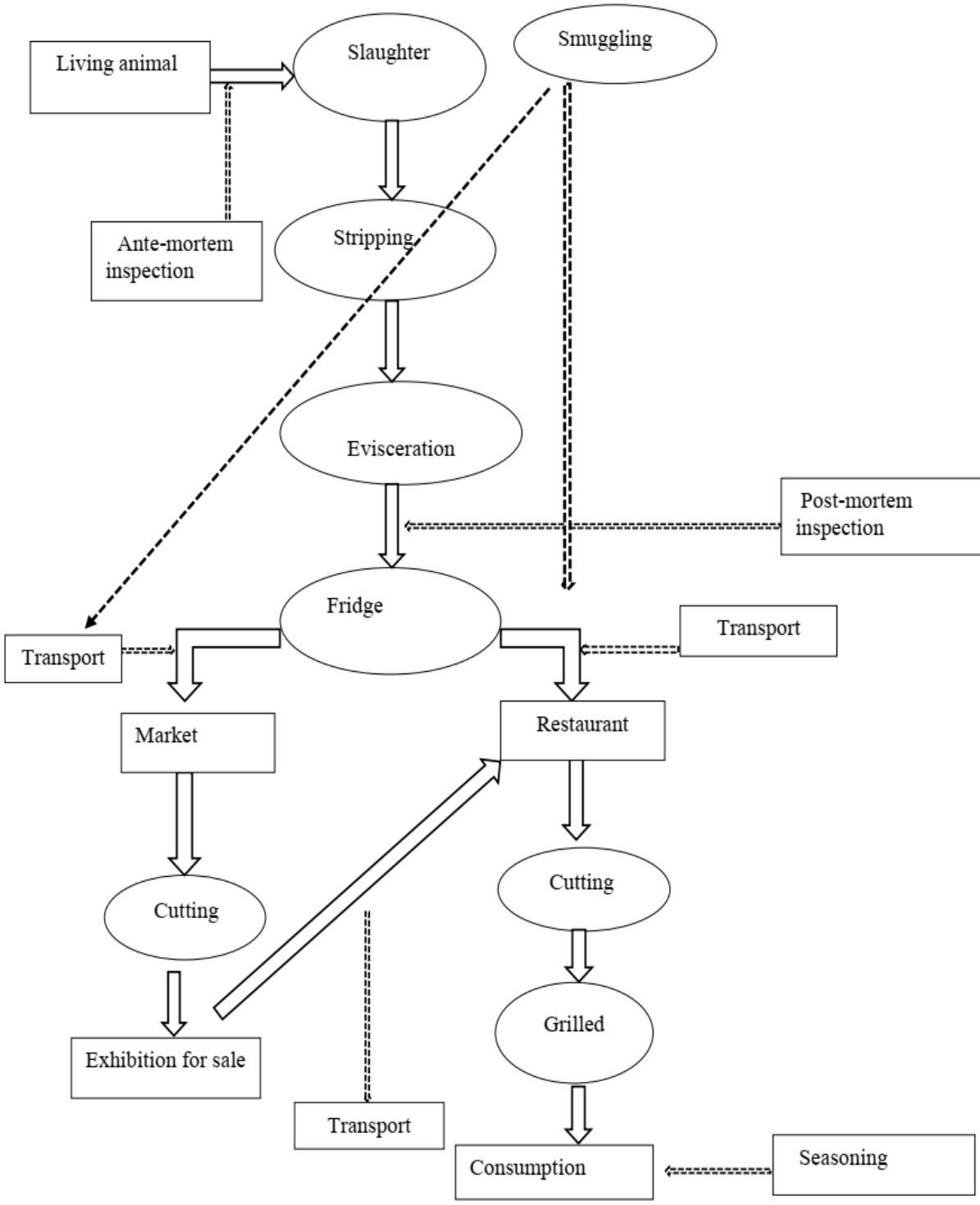

Figure 1: Meat supply chain.

Table 3: Microbiological quality of grilled meat collected from vendors in 06 districts of N'Djamena.

Code	TMAF (CFU/g)	TC (CFU/g)	TTC (CFU/g)	*Salmonella* spp. (25 g)
A1	1.49 x 10^7	7.41 x 10^4	8.12 x 10^3	Absence
A2	1.45 x 10^7	3.18 x 10^5	1.55 x 10^4	Absence
A3	1.85 x 10^7	3.90 x 10^5	9.37 x 10^4	Absence
A4	1.81 x 10^7	7.12 x 10^5	7.77 x 10^4	Absence
A5	1.31 x 10^7	6.64 x 10^5	3.86 x 10^4	Absence
A6	1.11 x 10^6	8.11 x 10^4	1.30 x 10^3	Absence

Legend: TMAF: Total Mesophilic Aerobic Flora, TC: Total Coliforms, TTC: Thermotolerant Coliforms, CFU: Colony Forming Unit.

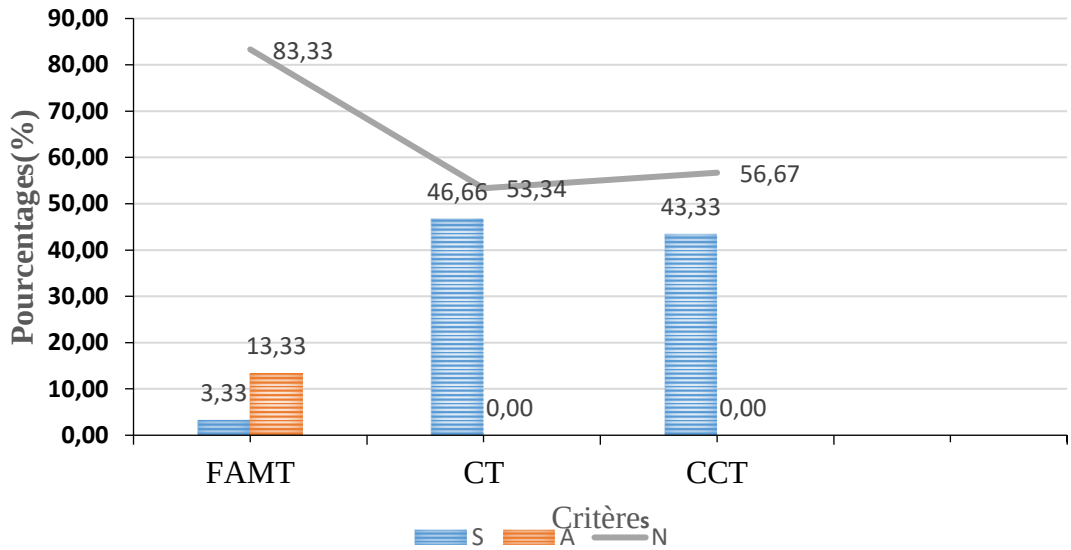

Figure 2: level of contamination of grilled meat by total aerobic mesophilic flora and coliforms.
S: Satisfactory, A: Acceptable, N: Not Satisfactory, TMAF: Total Mesophilic Aerobic Flora, CT: Total Coliforms, CTT: Thermotolerant Coliforms.

Table 4: Susceptibility of *Escherichia coli* Strains to Antibiotics.

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
NOR	n= 11 (100)	0	0
IMP	n= 11 (100)	0	0
NA	n= 3 (27. 27)	n= 4 (36. 36)	n= 4 (36. 36)
CN	n= 5 (45. 55)	0	n= 6 (54. 55)
CRO	n= 3 (27. 27)	n= 1 (9. 09)	n= 7 (63. 64)
CEF	0	n= 3 (27. 27)	n= 8 (72. 73)
SXT	n= 4 (36. 36)	n= 5 (45. 45)	n= 2 (18. 18)
AMP	0	0	n= 11 (100)
AUG	n= 2 (18. 18)	0	n= 9 (81. 82)
CIP	n=11 (100)	0	0

Legend : NOR = Norfloxacin, IMP = Imipenem, NA = Nalidixic acid, CN = Gentamicin, CRO = Ceftriaxone, CEF = Cefotaxime, SXT = Trimethoprim -Sulfamethazole, AMP = Ampicillin, AUG = Amoxicillin + Clavulanic acid, CIP = Ciprofloxacin,

DISCUSSION

In Chad, as in most African countries, for cultural reasons, grills in restaurants are reserved for men. Our results corroborate those of Haziz et al. (2019) in Cotonou and Dakouri et al. (2019) in Côte d'Ivoire which showed 100% for grilled meat and 94% for tuna fish. This could be explained by the increased needs of men and the role of head of household to engage in the informal sector in order to meet family needs. As shown in table I, 66.67% of vendors did not go to school and 27.33% having the primary level of education. Similar observations were made in Côte d'Ivoire (Dakouri et al., 2019; Kone et al., 2022) also indicating that the majority of tuna fish sellers were illiterate. Dossou et al. (2018) found that almost half of 46.50% of the sellers of meat products sold on the streets have a level of education below or at most equal to the primary level. 90% of sellers have not received any specific training such as those from Lomé in Togo (70.1%) (Dossou et al., 2018). The lack of training of personnel in the field of hygiene could lead to a lack of knowledge of basic hygiene rules at the place of preparation and sale of food.

According to microbiological criteria 1441/2007 / CE, concerning the TMAF (3×10^5 CFU/g) and the coliforms (10^2 CFU/g) in the broiled meat, our samples have been heavily contaminated by the bacteria at 83.33%. This could be explained by the fact that these restaurants are located public roads, road guards and markets with a presence of dust and grains of sand. Waste and open culverts located near these restaurants attract flies and insects responsible for the transmission of microorganisms (Wognin et al., 2022). Similar studies in Côte d'Ivoire have shown a high level of contamination (98%) in braised beef samples "*Choukouya*" (Dibi et al., 2017). However, our results were far superior them results obtained by Somda et al. (2018) and Kaboré et al. (2018) who obtained 38.24% and 60% respectively for roast/smoked chickens and grilled beef in Burkina Faso. This high level of contamination could be linked to non-compliance with hygiene rules when handling grilled meat and also to unhealthy sales premises. Waste water and garbage are often discharged or disposed of close to the service, thus making the environment very unhealthy. The behaviour of some vendors who scratch, cough and sneeze next to grilling and wearing dirty work clothes may also justify charges of non-compliance.

The poor condition of the equipment and the lack of rigor of the official control services may also explain the lack of hygiene. Studies by Benaissa et al. (2014) and Ilboudo et al. (2016) have shown that hygiene breaches are responsible for contamination values that exceed acceptable limits. Another study by Salifou et al. (2013) showed that the lack of regular disinfection of the equipment used, the non-observance of the rules of personal hygiene, clothing and behavioural manipulators are at the origin of contamination.

Thermo-tolerant coliforms were identified with a non-compliance rate of 56.67%. Authors such as Bennan et al. (2016) and Dossou et al. (2018) had obtained higher non-satisfaction rates respectively 66.12% and 26.70%. The thermotolerant coliforms are germs testifying the lack of hygienic observation. Their presence in food indicates human-induced contamination (Ilboudo et al., 2010). This could be due to the use of plates often washed with contaminated dirty water in the facility or subsequent contamination after treatment. A 2016 study in Chad reported that contaminated water is a public health risk when used for washing dishes (Tidjani et al., 2016). This finding is consistent with the work of Barro et al. (2006), who reported that bacteria in dirty dishwater and other sources adhere to the surface of utensils and pose a risk in the process of selling and preparing food. There is also poor equipment maintenance and lack of an effective procedure for cleaning-disinfecting utensils during processing and sales. Hand hygiene, the behaviour of staff who handle meat and money at the same time, the unjustified displacement of staff are factors that favour the contamination and proliferation of germs. The person handling money should not handle food. This is because money is dirty and can contaminate safe food (Tidjani et al., 2013). Several studies have shown that hands carry most of the time faeces contamination germs

that are often responsible for diarrheal diseases and gastroenteritis (Baba-Moussa et al., 2006; Bessimbaye et al., 2013). Other research has found that slicers and cutters are recognized as important vehicles for the contamination of cooked meat products both in the factory and at the point of sale (Pérez-Rodriguez et al., 2007).

No *Salmonella* spp. was isolated in our samples. Other authors have also reported the absence of *Salmonella* spp. on the samples of grilled meats (Dana et al., 2019; Dossou et al., 2018; Somda et al., 2018). Kaboré et al. (2018); Bennan et al, (2016) had shown the presence of *Salmonella* spp. in grilled meat in Burkina Faso and in beef and poultry products in Morocco with 20% and 25% respectively. This difference could be explained either by cross-contamination (ingredients, cutting equipment and personnel) or at a cooking time that is too short. On the other hand, this absence could be due to either the possible presence of competing germs, to the nature of the medium used for the isolation or to the efficiency of the cooking temperature. The absence of *Salmonella* spp. is a guarantee of food security (Baba-Moussa et al., 2006).

Escherichia coli isolates tested for resistance to 11 antimicrobials showed high levels of resistance to ampicillin (100%), amoxicillin + Clavulanic acid (81.82%), ceftriaxone (55%), gentamicin (55%) and cefotaxime (73%). Several studies on meat products have also reported the resistance of strains to beta-lactams (Siddiq et al., 2015, Zoubair et al., 2016, Somda et al., 2018). This resistance may be due to the accessibility and overuse of antibiotics in human and veterinary medicine (Thioune et al., 2022). Furthermore, the most marked resistance was observed with ampicillin (100%). Authors from different countries have detected considerable proportion of Ampicillin-resistant *E. coli* strains on meat products in Burkina Faso (Somda et al., 2018) (42.86%); Algeria (Bechiri et al., 2020) ((99.5%) and India (Jenifer1 & Sathiyamurthy, 2020) (49%). Resistance to ceftriaxone, gentamycin and ampicillin is quite disturbing, as these molecules are molecules of last resort. They are also accessible in unity, and at affordable cost. In addition, Bessimbaye et al. (2013) point out that the increase in resistance is due to abusive and inappropriate prescription by health workers. For Armengaud et al. (1994), beta-lactam-resistant bacteria produce beta-lactamases that modify or inactivate antibiotics. This phenomenon of antibiotic resistance is a major public health problem that requires particular attention by the actors.

Conclusion

It appears from this study that most grilled meat samples have germs that exceed the microbiological criteria. The presence of these germs shows that grilled meat are handled in poor conditions and especially in often unhealthy environments by all staff. However, we did not isolate any *Salmonella* spp. isolates in our samples. Isolated bacteria were for the most part resistant to the beta-lactam family. This phenomenon could be due to the uncontrolled use of antibiotics which has led to a very worrying emergence of increasingly resistant bacteria. In other words, vigilance should be given to the use of medicines in the health and veterinary fields, to avoid the increase in resistance.

COMPETING INTERESTS

The authors declare that there is no competing interests.

AUTHORS' CONTRIBUTIONS

DE, NSS, and AAD conceived and designed the study. Collected and analysed the samples for the writing of the first draft was carried out by DE. NSS and HC had reviewed the manuscript. YEH, AT and AS had supervised and approved the final manuscript as submitted.

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