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Campylobacter spp Co-contamination of Meat Sold in Butcheries and Their Multidrug Resistance in Ngaoundere Municipality

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Abstract

Antibiotics resistance (ABR) is a major global public health problem. However, emerging hospital and community-based data indicated a rise in the prevalence of antibiotic resistance in developing low and middle-income countries, A total of 125 composite meat samples were collected from slaughter houses and different retail points during the dry and rainy season in the Municipality. Isolation of the specific pathogens was carried out using different traditional microbiology techniques. Antibiotics sensitivity test was conducted using Kirby Bauer disk diffusion technique. Results indicated a total of 218 isolates were obtained during the rainy season, against 124 isolates for the dry season. However, there was no statistical significant difference (P = 0.7) in the frequency of isolation between the two seasons .Campylobacter spp that were highly targeted were more frequently isolated in the rainy season. Antibiotic resistance profile varied from 42.6% in Vibrio spp to 73.4% in Salmonella spp. Microbial resistance index was as high as 0.7, indicating feco-oral route as the main source of meat contamination. The isolated bacteria were resistant to different antibiotic families of at different proportions. Our results are indications that contamination of beef with different food-borne pathogens constitutes serious problems for consumers, and therefore, the authorities in charge of food sanitation in general should seek for measures to ensure food safety for the exposed population.

Keywords: *Campylobacter spp, Vibrio spp, microbial resistance, MARI-not found in the abstract*
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1. Introduction

Antibiotic resistance (ABR) is a serious global health issue. Drug resistance raises the risk of disease transmission, severe illness, disability, and death by taking antibiotics and other antimicrobial treatments ineffective and making infections difficult or impossible to treat [1]. The emergence of antimicrobial resistance poses a severe threat to public health, impeding progress in the management of cancer, organ transplantation, infectious disorders and critical care. Furthermore, drug-resistant illnesses harm the health of both livestock and crops, impair farm productivity, and affect food security [2]. Microbiologists and infectious disease specialists have long recognized the problem. The discovery of penicillin by Sir Alexander Fleming drew attention to the threat of resistance from, but the realization of the vast scale of the resistant threats is only now reaching wider audiences [3]. Many infectious agents that could once be successfully treated with any one of several drug classes have acquired resistance to most microorganisms[4]. Foodborne diseases caused by *Campylobacter* spp, *Salmonella* spp, *Staphylococcus aureus,* and *Vibrio* spp constitute some of the major causes of mortality and infections, especially in developing countries [5]. These pathogens are transmitted mainly through the consumption of contaminated food, and the presence of these organisms in meat and raw meat products has relevant public health implications [6].. The prevalence of *Salmonella* varies from one region to another [7], and has been reported to cause a wide range of food and waterborne diseases both in humans and animals. The acquisition and spread of resistant genes are significantly affected by the exchange between plasmids and the bacterial chromosome, as well as the integration of resistant genes into specialized genetic components such as integrons [8]. *Campylobacter*, a foodborne bacterial pathogen, is the leading cause of human gastroenteritis worldwide [9]. The United States Center for Disease Control (CDC) indicated that almost 24% of *Campylobacter* strains tested were resistant to ciprofloxacin (fluoroquinolone) or azithromycin (macrolide), indicating that approximately 310,000 *Campylobacter* infections are caused by drug-resistant *Campylobacter* each year in the United States [9]. *Campylobacter* has evolved various mechanisms of resistance to antimicrobials, some of which confer resistance to a specific class of antimicrobials, while others may confer multidrug resistance [10]. *Campylobacter* is a major foodborne pathogen, and its resistance to clinically important antibiotics is increasingly prevalent. Rising fluoroquinolone resistance, particularly in *Campylobacter,* has been reported in many countries [11], limiting its use for the treatment of Campylobacteriosis.

Vibrio cholerae is a comma-shaped bacterium autochthonous to the aquatic environment that infects humans

through contaminated water or food, and is the causative agent of cholera, a self-limiting acute diarrheal disease. Over the years, several antimicrobials such as tetracycline, fluoroquinolones, and azithromycin have been effectively used in the treatment of cholera patients [12]. However, in the recent years, treatment failures are often seen with the recurrent emergence of antimicrobial resistant *V. cholerae* [13]. The development of resistance to many antibiotics by *S. aureus* has involved acquisition of determinants by horizontal gene transfer of mobile genetic elements [14]. These determinants may have evolved in antibiotic producers to protect them from potentially inhibitory molecules, or in their competitors. Analysis of the soil resistome shows that bacteria that express resistance to antibiotics are wide spread [15]. Because antibiotic exposure in healthcare (humans), agriculture (animals, plants, or food-processing technology), and the environment (sea, soil, drinking water, and wastewater) drives the development of antibiotic resistance, studies on the interactions between humans, animals, and the environment, as well as between the various sectors involved are crucial. Multidrug resistance (MDR) is a common problem that hurdles chemotherapy. To overcome this problem, it is a prerequisite to identify the multidrug resistance pattern of bacteria isolates. As such, this study was investigated to isolate *Campylobacter* spp, *Salmonella* spp *Staphylococcus aureus* and *Vibrio cholera* from retailed meat sold in Ngaoundere municipality; in order to determine their MDR pattern and MAR Index, through submitting the isolates to different antibiotics

Methodology

2.1 Study site

The study was carried out in the Vina division, located in latitude 7,20° and longitude13,50° At a height of 1142 meters above sea level.

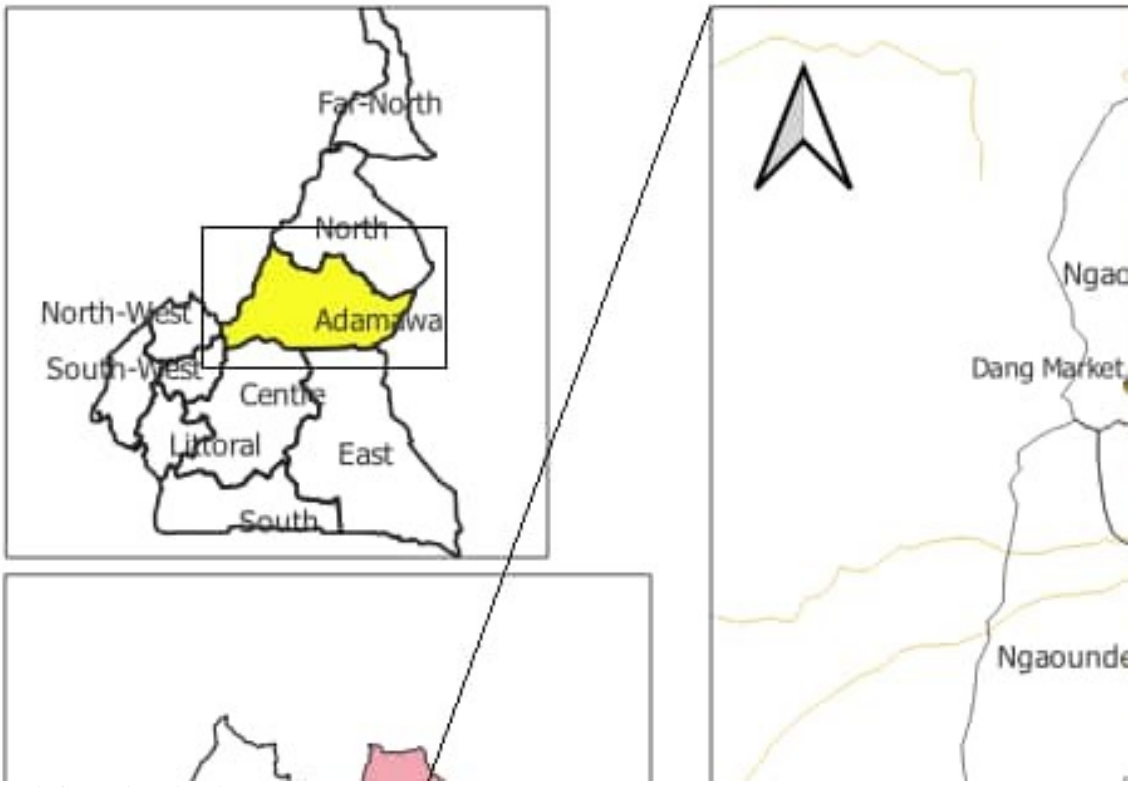

Figure 1. Sampling sites in the study area

Figure 1 gives positions in the different council areas where sampling took place**.**

2.2 Sample collection and isolation of bacteria

A total of 125 composite (total of 500 samples) samples were collected from butcheries and in abattoirs in different sale points and markets of the Ngaoundere municipality in the dry and rainy season for a period of two years. Characterization and identification were conducted using conventional methods in microbiology controlled by Gram staining and confirmed using Analytical profile index test kit purchased from bioMerieux.

2.3 Isolation of bacteria

Different selective culture media were used to isolate some pathogenic bacteria presumed to be found in meat samples. Charcoalcefoperazone deoxycholate modified agar base (lot25451/ 62026) was prepared according to manufacturer's instructions and used in the isolation of *Campylobacter* spp. Xylene Lysine Deoxychocolate agar (XLD20500) was also prepared according to the manufacturers directives and used in the isolation of *Salmonella* spp, *Stahphylococcus* spp, while *Micrococcus* spp were isolated using Mannitol salt agar(90150689) prepared accordingly and differentiated by Gram staining. *Vibrio* spp were isolated using Thiosulphate Citrate Bile salt agar (ref90101424 prepared as described by the producer. Enteropathogenic. *Escherichia coli* was isolated using MacKonkey Sorbitol to help in the identification of pathogenic *E.coli*. Different species of bacteria isolated were then confirmed by using Analytical Profile Index test system (20100, bioMerieux). Meanwhile, *Aspergilus* spp was isolated using Sabouraud Dextrose agar (Harmonised) ref 90105391.

2.4 Antimicrobial susceptibility test

The antimicrobial susceptibility test was performed using the disc-diffusion method as recommended by Clinical Laboratory Institute Standards [16]. Bacterial isolates were grown for 18 hours on nutrient agar. They were suspended in 2 ml sterile normal saline and turbidity adjusted to match McFarland Opacity Standard No0.5 (equivalent to 1.5 x 108 bacterial density). Bacterial suspensions of 0.1 mL were dispensed on the surface of sterile Mueller-Hinton agar plate and spread evenly using a sterile spreader. This was allowed to dry for 5 minutes, before antibiotic discs were dispensed on the surface of the media and incubated aerobically at 37^{o}C for 18 hours. The susceptibility patterns of the isolates to different antibiotics were noted as Sensitive (S) or Resistant (R), as per CLSI standards [16]. Intermediate-resistance was taken as full-blown resistance. The following antimicrobial agents (single discs, Oxoid Ltd., Basingstoke, Hamphire, England) were tested**.** Ampicillin (AMP 10µg), Amoxicillin-clavulanic acid (AMC 20 + 10 µg), cefoxin (FOX 30ug), Imepenem (IPM10ug), Cefepine (FEP30), Methycillin (Mer5 µg), gentamicin (GEN 10 µg), amikacin(AK 30 µg), chloramphenicol (CHL30 µg), Tetracycline (TE 30µg) Doxyciclin(DO 30ug),Colistrine(COL10ug) , Nalidixic acid (NAL 30 µg), ciprofloxacin (CIP 5µg), Triméthoprime Sulfamethoxazole(SXT 1,25 + 23,75 µg) Spiramycin (SP 30), Penecillin (G 10µg), Neomycin(N 30ug), Ceftazidime(CAZ 30), Pefloxacin(PEF 5ug), Cloxacillin (CX 5ug) Norfloxacin(NOR 5ug).

Table 1: Functional groups of antibiotics used

Families	Antibiotics
Bêtalactamase	AMP= Ampicilline
	AMC=Amoxicilline + clavulanic acid
	FOX= céfoxin
	IMP= imipenème
	FEP= Céfépime
	MET= Methiciline
	CAZ= ceftazidime
	P= Penecilin
	Neo = Neomycin
Aminoglocosides	GEN=gentamycine ;
	AKN=amikacine
Phénols	CHL=chloramphénicol
Cyclines	TET=tétracycline
	DO = Doxycicline
Polypeptides	CST=colistine
Quinolones	CIP= ciprofloxacine
	NAL= Nalidixic acid
	NOR= norfloxacine
	PEF= Pefloxatine
Diaminopyrimidines **Sulfamides**	SXT=Triméthoprime/sulfaméthoxazole
Macrolides	SP = Spiramycine

2.5 Identification of Multidrug Resistance (MDR) Strains

The number of antibiotics each bacterium was resistant to in the disc diffusion test was noted for identification of multidrug resistant strains. Multidrug resistance (MDR) was taken as resistant to four or more antibiotics tested on the different bacteria isolated [17].

2.6 Calculation of Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance (MAR) index was calculated as **a/b,** where **a** represents the number of antibiotics to which the isolates were resistant and '**b**' represents the total number of antibiotics to which the isolate was exposed [18].

Statistical analysis. Statistical analysis was carried out using excel to draw the frequency charts and T test to compare the frequencies of isolation of different pathogenic agents in the dry and rainy season.

3 Results

Of 125 composite samples (a total 500 samples), in the two seasons 39.2% were positive for *Campylobacter* spp, 41.6% positive for *Salmonella* spp , 46.6 % positive for *Staphylococcus* spp, 32% for *Micrococcus* spp , 41.6 positive for *Vibrio* spp, 32% for Enteropathogenic *E. coli and* 28% for *Apergilus* spp 20%

Table 2. Etiology of some enteric pathogens isolated during the dry and rainy seasons

Etiological agent		Numbder isolates	Percent isolation		Number of isolates	Percent isolation
Campylobacter spp		29	13		20	16.1
Salmonella spp		34	15,6		18	14.5
Stahphylococcus spp,		45	20.6		23	18.5
Micrococcus spp		27	12.4	Dry Season	19	15.3
Vibrio spp	Rainy Season	34	15.6		18	14.5
Enteropathogenic *Escherichia.coli*		26	11.9		14	11.2
Aspergilus spp		23	10.6		12	9.7
Total isolates per season		218 isolates			124	

Table 2 indicates that a total of 218 isolates were obtained in the rainy season, while 124 were isolated in the dry season. *Staphylococcocus* spp, *Salmonella* spp, *Vibrio* spp were the most abundant (expressed in percentages) of bacteria isolated in the rainy season, whereas in the dry season, it is instead *Staphylococcus* spp, *Campylobacter* spp, and *Micrococus* spp that were the highest percentages.

Table 3: Resistance pattern of bacteria strains to different antibiotics

Serial number	Bacteria isolates	Frequency of bacteria	Resistance (%)	Susceptible phenotypes	Resistant Phenotypes
1	*Campylobacter* spp	49	61.9	(AMP 10µg), GEN 10 µg), AKN 30 µg), (TE 30µg) DO30ug), NAL 30 µg), SXT 1,25 + 23,75 µg), CAZ 30), AMC	CX 5ug) (AMC 20 + 10 µg), FOX 30ug), IPM10ug), FEP30ug, MET5 µg), , CHL30 µg), (TET 30µg) COL10ug), CIP 5µg), SXT (SP 30) (P10µg), (N 30ug, NOR 5ug).
2	*Salmonella spp*	52	71.4	GEN 10 µg), AKN 30 µg), DO30ug), COL10ug), NAL 30 µg), CIP 5µg),	(AMP 10µg), (AMC 20 + 10 µg), FOX 30ug), IPM10ug), , MET5 µg), GEN 10 µg), CHL30 µg), (TET 30µg) SXT 1,25 + 23,75 µg) (SP 30) (P 10µg), (N 30ug, CAZ 30), NOR 5ug). CX 5ug) FEP30ug, AMC
3	*Stahphylococcus spp*	68	57.1	(AMC 20 + 10 µg), MET5 µg), GEN 10 µg), AK 30 NAL 30, CAZ 30), NOR 5ug). CX 5ug) AMC	(AMP 10µgFOX 30ug), IPM10ug), FEP30ug, , GEN 10 µg), CHL30 µg), (TET 30µg) DO30ug), COL10ug),

					CIP 5µg), SXT 1,25 + 23,75 µg) (SP 30) (P10µg), (N 30ug, CAZ 30).
4	*Micrococcus spp*	46	61.9	(AMP 10µg), (AMC 20 + 10 µg), FEP30ug, MET 5 µg), DO30ug), NAL 30 SXT 1,25 + 23,75 µg) NOR 5ug). AMC	FOX 30ug), IPM10ug), GEN 10 µg), AKN 30 µg), CHL30 µg), (TET 30µg) COL10ug), CIP 5µg), µg) (SP 30) (P10µg), (N 30ug, CAZ 30),
5	*Vibrio spp*	52	42.9	(AMP 10µg), (AMC 20 + 10 µg), FOX 30ug), IPM10ug),), GEN 10 µg), , DO30ug), COL10ug), NAL 30 µg), CIP 5µg), SXT 1,25 + 23,75 µg) (SP), CAZ 30), AK 30 µg),	FEP30ug, MET5 µg), CHL30 µg), (TET 30µg) COL10ug), , CIP 5µg (SP 30) (P 10µg), (N 30ug,), NOR 5ug). CX 5ug) AMC
6	Enteropathogenic *Escherichia coli*	40	52.4	(AMP 10µg), (AMC 20 + 10 µg), IPM10ug), GEN 10 µg),), COL10ug), NAL 30 µg), CIP 5µg), SXT 1,25 + 23,75 µg), CAZ 30), AMC	(FOX 30ug), FEP30ug, MET5 µg), GEN 10 µg), AKN 30 µg), CHL30 µg), (TE 30µg) DO30ug), CIP 5µg), (SP 30) (P10µg), (N 30ug, NOR 5ug). CX 5ug)

In table 3, the isolation frequency of *Salmonella* spp was 52, and they were resistant to 71,4% of different antibiotic families used, giving them the highest resistance profile. This was followed by *Campylobacter* spp and *Micrococcus* spp with 61.9% of resistance each still cutting across the different antibiotic families. Meanwhile, *Vibrio* spp had a frequency of 52 and were resistance to 42.9% of the antibiotics involving different families. All the bacteria isolates indicated the presence of multidrug resistance genes in this test

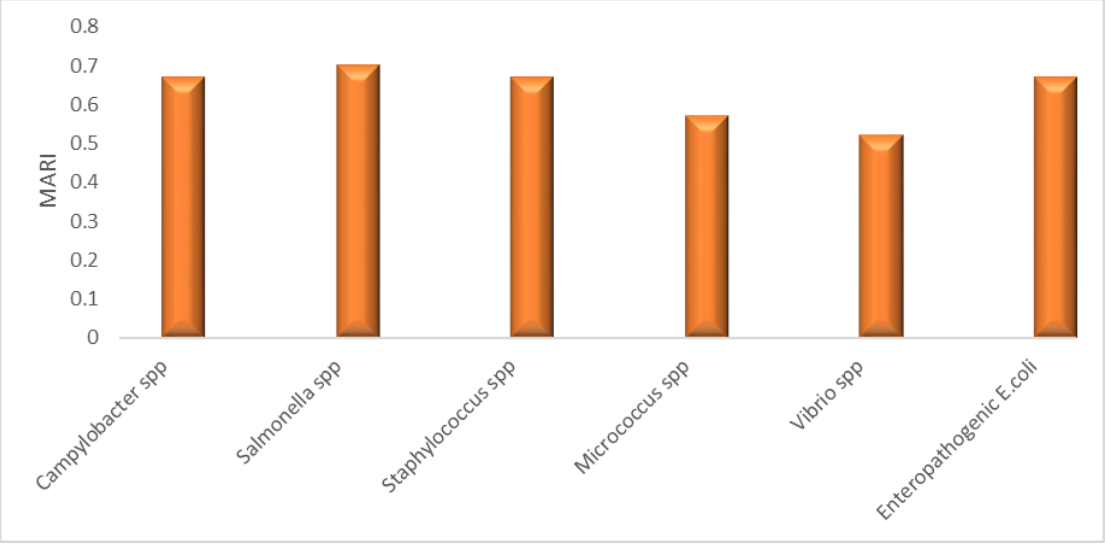

Figure 2: **Multiple antibiotic resistance indices of different isolates**

Figure 2 indicate vriable MARI of different bacteria isolated in the dry and rainy season as high as 0.7. High MAR indices mandate vigilant surveillance and remedial measures.MAR index of 0.2 and above is worrisome. Sensitivity patterns and treatment must be guided by laboratory investigations

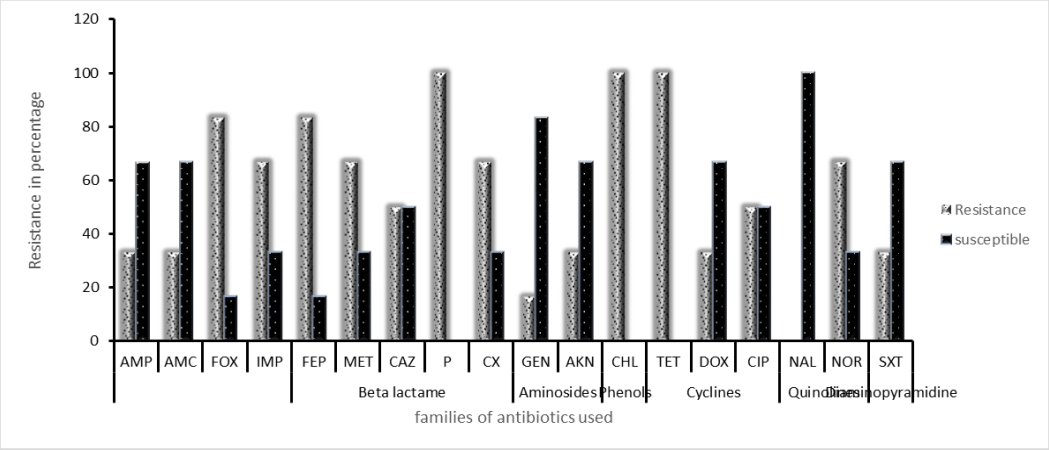

Figure 3: Antibiotic resistance pattern of different isolates

Figure 3 shows the resistance to various antibiotics families tested. The analysis of the antibiotic resistance pattern of these pathogens revealed that the resistance pattern of different antibiotics families to various isolates obtain from P, FEP, FOX constituted the highest resistance from the betta lactam family. Isolates were also highly resistant to aminoglycosides, represented by Chloramphenicol. Bacteria were equally very resistant to Tetracyclines which belongs to the cyclines group of antibiotics. Bacteria isolates were more than 60% susceptible to AMP and AMC in the beta lactamase family. Whereas isolates were 65% susceptible to aminoglycosides made of GEN and AKN, Susceptibility of the isolates was also demonstrated in the family of quinolines by NAL**.**

DISCUSSION

Surveillance of antimicrobial resistance and antimicrobial use in both human and nonhuman sectors is necessary to estimate the extent, patterns, and health burden of resistance at the national, regional, and international levels. Food borne diseases caused mainly by *Campylobacter* spp. *E. coli*, *Salmonella* spp and *Staphylococcus aureus* are some major causes of mortality and infections especially in the developing countries. These pathogens are transmitted mainly through consumption of contaminated food and the presence of these organisms in meat and raw meat products has relevant public health implications [6]. In this study, we observed a trend in the etiological agents causing meat contamination. Detection of these etiological agents is important for all therapeutic aspects and for implementing appropriate sanitation strategies in our food distribution centers especially in local butcheries to curb the spread of diseases. Equally, this study revealed different types of bacteria (*Campylobacter, Salmonella*, *Staphylococcus Micrococus Vibrio* sp , Enteropathogenic *E.coli* and *Aspergillus* spp responsible for the contamination of meat. This is in line with studies by Fahim *et al*,(2016) [19] on the isolation of etiological agents in meat and meat products in Benha. *Campylobacter* spp is a major foodborne pathogen, and its resistance to clinically important antibiotics is increasingly prevalent. This study has indicated that *Campylobacte*r spp isolated were 61.9% resistant to antibiotics. Resistance to four or more antibiotics was confirmed as multidrug resistant strains. Multiple drug resistance has become a common feature of many microorganisms, especially the human pathogens. Data obtained from this study is however much similar to that obtained by Shen *et al*., (2019) [20] during the study of antimicrobial resistance in C*ampylobacter* spp in Copenhagen. This bacteria was susceptible to AMP (10µg), GEN (10 µg), AKN (30 µg), (TE (30µg) DO (30ug), NAL (30 µg), SXT (1,25 + 23,75 µg), CAZ (30 µg), AMC, in agreement with studies conducted by Kanako *et al*.[21], who determine the contamination of retail chicken by *Campylobacter* in Japan.

Salmonella is globally one of the leading causes of human death among diarrheal diseases. Understanding the epidemiological status of *Salmonella* is thus crucial for controlling this pathogen [22]. In our analysis, *Salmonella* had a percentage resistance of 71.4 % (AMP 10µg), AMC (20 + 10 µg), FOX (30ug), IPM (10ug), MET (5 µg), GEN (10 µg), CHL (30 µg), TET (30µg) SXT (1,25 + 23,75 µg) SP (30), P (10µg), N (30ug), CAZ (30 ug), NOR (5ug). CX (5ug) FEP (30ug), cutting across different group of antibiotics used in this exercise. This is an indication that *Salmonella* spp possess multi drug resistance properties. This is in line with studies conducted by Tangwa *et al.*[23] in the study of antibiotics resistance of some bacteria isolated from water samples in Ngaoudere. In contrast, *Salmonella* spp was susceptible to some antibiotics used in the exercise.

Staphylococcus aureus isolates from different sources in many parts of the world are increasingly resistant to a greater number of antimicrobial agents [24]. Results obtained from this study indicated that the isolation frequency of *Staphylococcus* spp was 68 and gave a resistance pattern of 57.1% cutting across different families of antibiotics used. This finding confirms the very high prevalence of Antibiotic resistance threats of *Staphylococcus* and other bacteria species reported in the United States [9].

Emergence of MDR and XDR in *Vibrio cholerae* is an excellent example of bacterial evolution in the recent time. In our study, *Vibrio* spp demonstrated a good example of multi drug (42,3%) resistance to different families of anitibiotics that were applicable in this study though it were lowest compared to bacteria isolated. Studies published in India demonstrated similar results [25].

Vibrio spp was also susceptible to a numder of antibiobiotics across the board as indicated by Marin *et al*. [26], indicating the worldwide, occurrence of integrative conjugative elements encoding multidrug resistance determinants in epidemic *Vibrio cholera.*

According to Mishra *et al*. [27], MAR index of 0.2 or higher indicates high risk sources of contamination, while MAR index of 0.4 or higher is associated with human faecal source of contamination. Thenmozhi *et al*. [28] stated that MAR index values > 0.2 indicate existence sources with frequency use of antibiotics, while values ≤ 0.2 show bacteria from source with less antibiotics usage. In this study, all the isolates had MARI far above > 0.2, and as such, meat selling points in Ngaoundere mandate vigilant surveillance and remedial measures to avoid the rapid spread of resistance bacteria to antibiotics commonly used in this area.

The pathogens isolated were resistance to different families of antibiotics used. β-Lactam family, the class of antibiotic agents that contains a β-lactam ring in their molecular structures, was used in this study and the results indicated that the isolates were resistance to P, FEP, FOX, MET and CX respectively, while others were susceptible to AMP, AMC, CAZ and CX. This piece of work is similar to that published by Jovetic *et al.* [29] who indicated in their write up.

Aminoglycosides were generally considered to have broad-spectrum bacteriocidal activity. In this study GEN and AKN were used and the isolates were more resistance to GEN than AKN. Meanwhile, bacteria were more susceptible to AKN than GEN, similar to previous published work by Ramirez and Tolmasky [30]. Nonfluorinated or fluorinated phenols which are highly effective against a wide variety of Gram-positive and Gram-negative bacteria used in this study was Chloramphenicol, to which all the species of isolated bacteria were highly resistant. Similar to this result, Blaser and Engberg [31] have shown a rising prevalence of resistance of *Campylobacter* and other enteric bacteria to phenol antibiotics.

For Tetracyclines which have broad-spectrum activity against Gram-positive and Gram-negative bacteria, used in this work were TET, DOX and CIP, isolates were 100% resistant to TET followed by CIP. In this family the highest susceptibility was obtained with DOX similar to studies published by Nguyen *et al.* (2014)[32].

Conclusion

This study demonstrated that meat contamination is public health hazard
and the presence of aerobic bacteria; *Campylobacter* spp; *E. coli Salmonella* spp S*taphylococcus* spp and *Vibrio* spp may be due to mishandling and the negligence of hygienic aspects either at production levels where most workers do not have medical certificates to sell meat. Given the highly nature of antimicrobial resistance revealed in this study, there is the propensity of resistance to spread between ecological niches in the human, animal, and environmental sectors. Therefore, we need to be more cautious. Antimicrobial stewardship programs should be aggressive in setting their targets to reduce antimicrobial use and also focus on those practices that are most obviously linked to the spread of antibiotic resistance such as sanitation our environment.

Competing interest

The authors declare that they have no competing interest.

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Limitations

Our limitations were mostly based on logistics, the attitude of some meat vendors who stopped us from sampling

and associated it to wish craft practices.

Author contributions

Author Contributions: **Conceptualization**: ALIYOU HAYATOU, **Methodology**: ALIYOU HAYATOU, TANGWA Bernard VIBAN and NGU NGWA Victor **Resources**: ALIOU HAYATOU and TANGWA Bernard VIBAN

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