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Research Article

One health concept, prevalence and phenotypic antibiotic susceptibility of *Escherichia coli* and *Salmonella* isolated from meats sold in Lagos, Nigeria

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Abstract

This study reports the one health concept, prevalence, and antimicrobial susceptibility patterns of *Salmonella* and *Escherichia coli* isolated from raw and ready-to-eat (RTE) meats sold in cities of Lagos State, Nigeria. The conventional method of isolation was used to isolate *E. coli* and *Salmonella* spp. on their respective selective media from fifty meat samples obtained from abattoirs, open display, and packaged products at various locations in the state and was confirmed by Gram's reaction and biochemical tests. Thirty-three *E. coli* and Twenty-seven *Salmonella* spp. were isolated with the overall prevalence rate recorded as 72% and 68% respectively. The isolated bacteria were subjected to antimicrobial susceptibility testing on nine different antibiotics using the agar disc diffusion method. All the *Salmonella* were resistant to at least one antibiotic while two *E. coli* isolates showed susceptibility to all the antibiotics used in this study. Of the 33 *E. coli* subjected to antimicrobial testing, 84.8% were susceptible to gentamicin, 81.8% susceptible to ciprofloxacin, and 75.8% susceptible to Augmentin. A lower susceptibility pattern was observed on *Salmonella* with 74.1% of the *Salmonella* being susceptible to ciprofloxacin and gentamicin, and 70.4% susceptible to azithromycin. Gentamicin was the most effective antibiotic while amoxicillin was found to be least effective against *E. coli* and *Salmonella* isolated from the meat samples used in this study. The multiple antibiotic resistance (MAR) index of *Salmonella* ranged between 0.11-0.67 while *E. coli* ranged between 0-0.89. *E. coli* was found to be more resistant than *Salmonella* and the bacteria isolated from RTE meats showed higher MAR than those isolated from raw meats.

Introduction

E. coli and *Salmonella* are Gram-negative bacteria belonging to the Enterobacteriaceae family that are commonly found in the intestinal tract of humans and animals [1]. These bacteria have been the causative agents of food poisoning, and urinary tract infections [2] and under severe conditions, they cause foodborne infections that could also lead to hemolytic colitis, hemolytic uremic syndrome, bacteremia, Reiter's arthritis, and death [3]. The impact of these pathogens results in the loss of longer shelf life of food and huge monetary loss to the farmers and the sellers of products [4]. The negative impact of these diseases on human and animal populations cannot be overestimated as various outbreaks of *E. coli* and *Salmonella* have been reported in different countries of the world [5]. According to WHO [5], of the total foodborne illnesses that occur in the world, 40% affect children under 5 years old leading to 125,000 deaths per annum. *Salmonella typhimurium* was reported to be responsible for 115 million infections in humans and 370,000 deaths in the world per annum [6].

Infections by these bacteria are caused by the consumption of contaminated raw and poorly cooked food, feed in animals, and equipment such as utensils at homes and farm tools. Meats such as beef, pork, mutton, chicken, and their products have been reported to play major roles in the transmission of *E. coli* and *Salmonella* that are responsible for foodborne illnesses. Various studies have reported the presence of *E. coli* and *Salmonella* in different meats and their products [4,7,8]. Likewise, studies

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have shown that the level of *E. coli* and *Salmonella* prevalence in meats varies between regions as regions with better hygiene practices and proper handling of food and food products have lower prevalence and foodborne illness outbreaks.

Food poisoning infections require the use of antibiotics to alleviate the effects in both human and animal populations; however, bacteria have developed resistance to large numbers of antibiotics in use; thereby posing challenges that must be overcome. Also, the emergence of cross-resistance among pathogenic bacteria has greater consequences too. Cross-resistance which has been reported in many bacteria including *E. coli* and *Salmonella* is a process that occurs when resistance to one antibiotic causes resistance to another antibiotic of the same or different classes [9]. This cross-resistance is caused by different mechanisms such as genetic mutations, target site alteration, horizontal gene transfer, and efflux pumps [9]. This resistance and cross-resistance to antibiotics are attributed to the misuse, overuse, and excessive application of antibiotics in animals and humans; thereby leading the bacteria to modify means and mechanisms to overcome the constraint. This development of mechanisms to overcome antibiotic pressure has led to failure in the treatment of various bacterial-caused infections in humans and animals. To overcome the problem of food poisoning, the adoption of measures such as education on proper handling and processing, good agricultural practices, adherence to HACCP in the slaughtering and processing of meats for consumption, and proper disposal of waste should be adhered to [3]. Attention should be more focused on the prevention and control of food, feed, and equipment contamination by both food poisoning microbes and non-food poisoning microbes to maintain healthy living and retain the quality of food. The farmers, doctors, and drug administrators should not only be concerned about healing and curing diseases but also the effects of the overuse, misuse, and prolonged application on the environment should be considered.

The way the food production system, humans, animals, and the ecosystem/environment in which we live are interconnected is a prime example of the One Health Concept. Foods from animals play important roles in providing humans and animals with proteins and micronutrients. These animals have been reported to be responsible (hosts) for three out of five human diseases: zoonotic diseases [10]. Unhygienic environments have also been found to be breeding habitats for some animals that harbor diseases causing pathogens. Thereby, the concept illustrates that the health of people is connected to the health of animals, plants, and our shared environment. To promote the healthy existence of humans, the health of plants and animals that humans depend on for food and interact with, and the environment in which they are found must be maintained. Another layer of complexity to this complex issue is the role that the environment, especially soil and water, plays as a reservoir for bacteria resistant to antibiotics [11]. Antimicrobial resistance pathogens spread from animals to humans through these resistant strains' ability to enter the human food chain. Since antimicrobial resistance must be addressed immediately, the One Health idea has been strengthened as infectious pathogens evade drugs meant to fight them. With the understanding that antimicrobial resistance is a complicated system woven across multiple fields, this holistic approach highlights the delicate relationship between the health of people, animals, plants, and the environment [12].

However, there is limited study and information available on the level of meat contamination and the spread of coliform, *E. coli,* and *Salmonella* among humans, animals, food, and environment in Lagos, Nigeria; whereby, there are loopholes in the handling before, during and after slaughtering of animals such as improper waste disposal, display over the counter, roadside selling, and inadequate heating were observed during this study. This study is based on the One Health viewpoint, the prevalence and phenotypic antibiotic susceptibility of *Salmonella* and *Escherichia coli* isolated from meats sold in Lagos, Nigeria. Through the evaluation of these bacteria's prevalence and antibiotic resistance in meat sold in Lagos, the research contributes to the One Health goal of addressing antimicrobial resistance from a multidisciplinary perspective [13]. Given the growing impact of antimicrobial resistance, addressing this issue requires a unified front. Our goal is to add to the body of information required for creating strategies to slow the spread of antibiotic resistance across human, animal, and environmental interfaces by adopting the One Health approach and concentrating on the antimicrobial resistance landscape [14].

Materials and methods

Location of the study

This study was carried out in various cities in Lagos state, Nigeria. The state is estimated to have a land size of 3,577 square kilometers which culminates to 0.38% of Nigeria's landmass. It shares boundaries with the Ogun state in the Northeast, the Bight of Benin in the south, and to the west the international border with the Republic of Benin.

Sampling of raw meats and Ready-to-Eat (RTE) meats for the study

In total, 50 meat samples were collected randomly and examined for the presence of coliform, *E. coli,* and *Salmonella.* These samples consisted of 5 each of raw chevon, raw guinea fowl, raw chicken, raw beef, raw mutton, RTE chevon, RTE guinea fowl, RTE chicken, RTE beef, and RTE mutton. Ten (10g) of each sample was collected in a sterile sampling bag (zip lock) and transported to the laboratory in a container containing ice packs. The samples were analyzed immediately in the laboratory.

Analysis of meat samples for coliforms, *E. coli* and *Salmonella*

Ten (10g) of the meat samples were pre-enriched in 90 ml peptone-buffered water and incubated at 37°C for 24 hours. 10ml of the homogenized culture was Pipetted into 90ml sterile distilled water to obtain 10^{-1} dilution. 10ml of 10^{-1} dilution was also transferred into another 90ml sterile distilled water to obtain 10^{-2} dilution. This procedure was repeated up to 10^{-4} dilution. For the culturing of raw meats aliquots, 0.1ml of the

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10^{-2} to 10^{-4} aliquot was inoculated into MacConkey agar plate by spread plate technique for coliform count enumeration, and 0.1ml of 10^{-2} dilutions were streaked on Levine's Eosin Methylene Blue Agar (EMB) and Xylose Lysine Deoxycholate agar (XLD) for *E. coli* and *Salmonella* detection respectively. For RTE meats, 0.1ml of the 10^{-1} to 10^{-3} aliquot was inoculated into Mac Conkey agar plate by spread plate technique and 0.1ml of 10^{-2} dilution was streaked on Levine's Eosin Methylene Blue Agar and Xylose Lysine Deoxycholate agar. These plates were incubated at 37°C for 24 hours in an inverted position [15].

Confirmation of *Escherichia coli* and *Salmonella* Isolated from raw meats and RTE meats

The colonies that appeared dark centered and flat, with or without a green metallic sheen on Eosin Methylene Blue Agar were isolated and pre-summed to be *E. coli*. The presumed *E. coli* was purified on trypticase soy agar and incubated at 37°C for 24 hours. These isolates were confirmed using the Gram staining technique and biochemical tests such as Methyl red, Voges Paskeur, indole, citrate, urease, and hydrogen sulfide tests [15]. Also, the colonies that appeared red with or without a dark center were isolated on Xylose Lysine Deoxycholate agar and presumed to be *Salmonella* spp. These colonies were further confirmed using Salmonella-Shigella agar, Gram staining technique, Methyl red, Voges Paskeur, indole, citrate, urease, and hydrogen sulfide tests [15].

Phenotypic antibiotic susceptibility testing

Antibiotic susceptibility testing was carried out on the isolates using the disk diffusion method according to the Clinical and Laboratory Standards Institute [16]. Thirty-three *E. coli* and twenty-seven *Salmonella* were isolated and subjected to antibiotic testing using the following antibiotics: ciprofloxacin 5µg (CIP), chloramphenicol 30µg (C), tetracycline 30ug (T), ceftriaxone 30µg (CRO), gentamicin 10µg (GM), amoxicillin 30µg (A), Augmentin 30µg (AUG), azithromycin 15µg (ATH) and trimethoprim 2.5µg (TM). Culture suspension was made by inoculating 24 hours pure young colonies into 5ml of sterilized normal saline contained in a test tube. The turbidity of the normal saline was adjusted to 0.5 McFarland standard turbidity. The standardized inoculum was inoculated into already prepared Mueller Hinton Agar by swab stick to obtain dense and uniform growth. Sterile Forceps was used to introduce antibiotic discs onto the surface of the inoculated plates and the plates were incubated at 37°C for 24 hours. After incubation, the inhibition zones produced by antibiotics against the inoculum were measured in mm using a ruler and interpreted by CLSI [17].

Multiple Antibiotic Resistance (MAR) index

The MAR index of each *Salmonella* and *E. coli* isolate was determined by the ratio of the number of antibiotics that a particular bacterium was resistant (a) to the number of antibiotics tested (b). MAR was then calculated as a/b. The multidrug resistance profile was taken as being resistant to 3 or more antibiotics used in this study [18].

Results

Prevalence of coliforms, *E. coli* and *Salmonella* in meats sold in Lagos, Nigeria

The examination of the fifty samples collected aseptically showed to be positive for coliform (100%), 72% for *E. coli*, and 68% for *Salmonella*. The highest coliform count was reported in raw meat and the least in ready-to-eat meat. On average, raw meats had a higher coliform count than ready-to-eat meats. Raw beef had the highest count of 7.3×10^{6} cfu/g and the lowest count on RTE chicken; 3×10^{2}cfu/g. The prevalence for *E. coli* and *Salmonella* ranged between 60-100% in all the meat samples. Guinea fowl was 100% positive for *E. coli* and *Salmonella*. In this study, 33 *E. coli* and 27 *Salmonella* were isolated from the samples positive for the respective bacteria. 22 and 11 *E. coli* were isolated from raw and RTE meats respectively and, 19 and 8 *Salmonella* from raw and RTE meats respectively Table 1.

Identification of the isolates by cultural methods

The bacteria were identified by the Gram staining method which showed that the isolates are Gram-negative. The biochemical test revealed *E. coli* to be positive for indole and methyl red test, negative for citrate, Voges Paskeur, hydrogen sulfide production, and urease tests. *Salmonella* showed positive results for methyl red and hydrogen sulfide tests, and negative for indole, citrate, Voges Paskeur, and urease tests Table 2.

Antimicrobial susceptibility pattern of *E. coli* isolated from meats sold in Lagos, Nigeria

In this study, 33 *E. coli* isolated from both raw and RTE meats were subjected to an in-vitro antibiotic test against

Table 1: Prevalence of *Escherichia coli*, *Salmonella* and Coliform counts of meat sold and eaten in Lagos, Nigeria.

Samples	No of samples Tested	Positive for coliform	coliform count range (log cfu/g)	% positive for *E. coli*	% positive for *Salmonella*
Chevon	5	5	4.5-6.7	80	60
Guinea fowl	5	5	4.2-6.5	100	100
Chicken	5	5	4.3-6.6	60	80
Mutton	5	5	4.5-6.6	60	60
Beef	5	5	4.6-6.9	80	60
RTE chevon	5	5	2.8-5.1	60	60
RTE fowl	5	5	2.7-5.3	80	60
RTE Chicken	5	5	2.5-5.2	60	60
RTE Mutton	5	5	3.3-5.4	80	80
RTE beef	5	5	2.2-5.2	60	60
	50	50		72	68

Table 2: Gram reaction and Biochemical tests characteristics of the *E. coli* and *Salmonella* Isolates.

Tests	Gram	Indole	Citrate	MR	VP	H_2S	Urease
E. coli	-	+	-	+	-	-	-
Salmonella	-	-	-	+	-	+	-

Key: H_2S: Hydrogen Sulfide

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nine antibiotics. The least number of resistances to antibiotics by *E. coli* was observed on gentamicin while the highest was reported on amoxicillin and trimethoprim (12 each) in raw meat. In this study, none of the 33 *E. coli* isolated from both raw and RTE meats was susceptible to amoxicillin. Gentamicin, azithromycin, and ciprofloxacin are the three most effective antibiotics that the *E. coli* isolates were highly susceptible to in this study. Two *E. coli* isolated from RTE mutton and raw beef showed resistance to all the antibiotics used in this study. Despite gentamicin being one of the most potent, none of the *E. coli* isolated from ready-to-eat meats was susceptible to its antibacterial action Table 3, Figure 1.

Antimicrobial susceptibility pattern of *Salmonella* isolated from meats sold in Lagos, Nigeria

Among the 27 *Salmonella* used for the antibiotic test, nineteen *Salmonella* were isolated from raw meats and eight from RTE meats. Unlike *E. coli*, all the Salmonella were susceptible to at least one of the antibiotics used in this study. In raw meat, the highest resistance was recorded on amoxicillin (13/19) followed by chloramphenicol (8/19), and the least resistance on gentamicin (1/19). In RTE meats, 4 of the 8 *Salmonella* were resistant to tetracycline and augmentin while 1 was resistant to trimethoprim. Unlike *E. coli* isolated from raw meats, *Salmonella* isolated from RTE meats showed high susceptibility to amoxicillin Table 4.

Phenotypic Antibiotic susceptibility profile of *Salmonella* isolated from meats sold in Lagos, Nigeria (Figure 2)

Table 3: Phenotypic Antibiotic susceptibility profile of *Escherichia coli* isolated from meats sold in Lagos, Nigeria.

	Raw Meats (*n* = 22)			RTE Meats (*n* = 11)		
Antibiotics	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Ciprofloxacin	13.6	9.1	77.3	18.2	9.1	72.7
Chloramphenicol	31.8	9.1	59.1	36.4	18.2	45.5
Tetracycline	27.3	13.6	59.1	54.5	9.1	36.4
Ceftriaxone	31.8	22.7	45.5	18.2	36.4	45.5
Gentamicin	9.1	0	90.9	0	27.3	72.7
Amoxicillin	54.5	45.5	0.0	72.7	27.3	0.0
Augmentin	77.3	13.6	9.1	63.6	18.2	18.2
Azithromycin	13.6	4.5	81.8	27.3	9.1	63.6
Trimethoprim	54.5	13.6	31.8	36.3	18.2	45.5

Table 4: Phenotypic Antibiotic susceptibility profile of Salmonella isolated from meats sold in Lagos, Nigeria.

	Raw Meats (*n* = 19)			RTE Meats (*n* = 8)		
Antibiotics	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Ciprofloxacin	10.5	26.3	63.2	37.5	12.5	50
Chloramphenicol	42.1	15.8	42.1	25	37.5	37.5
Tetracycline	15.8	15.8	68.4	50	12.5	37.5
Ceftriaxone	21.1	15.8	63.2	25	0.0	75
Gentamicin	5.3	15.8	79.0	25	12.5	62.5
Amoxicillin	68.4	10.5	21.1	37.5	0.0	62.5
Augmentin	10.5	26.3	63.2	50	12.5	37.5
Azithromycin	15.8	5.3	79.0	37.5	62.5	0.0
Trimethoprim	10.5	26.3	63.2	12.5	25	62.5

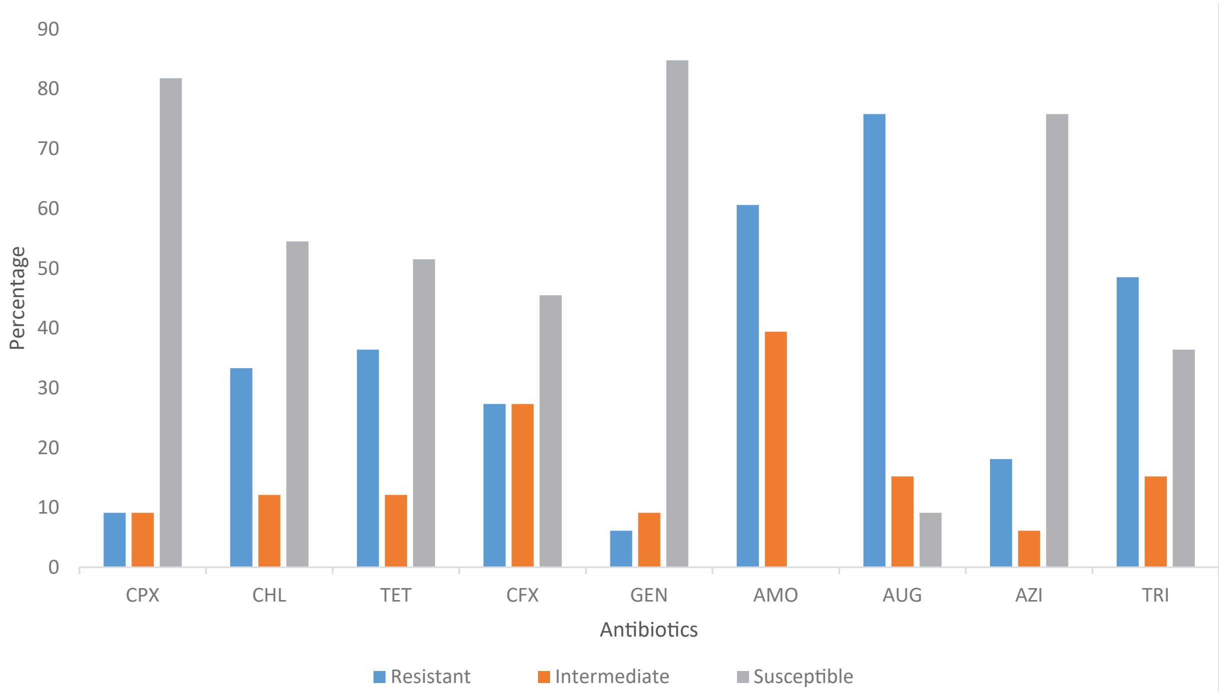

Figure 1: Overall antibiotic susceptibility profile of *Escherichia coli* isolated from meats sold in Lagos, Nigeria.
CIP: Ciprofloxacin; CHL: Chloramphenicol; TET: Tetracycline; CFX: Ceftriaxone; GEN: Gentamicin; AMO: Amoxicillin; AUG: Augmentin; AZI: Azithromycin and TRI: Trimethoprim

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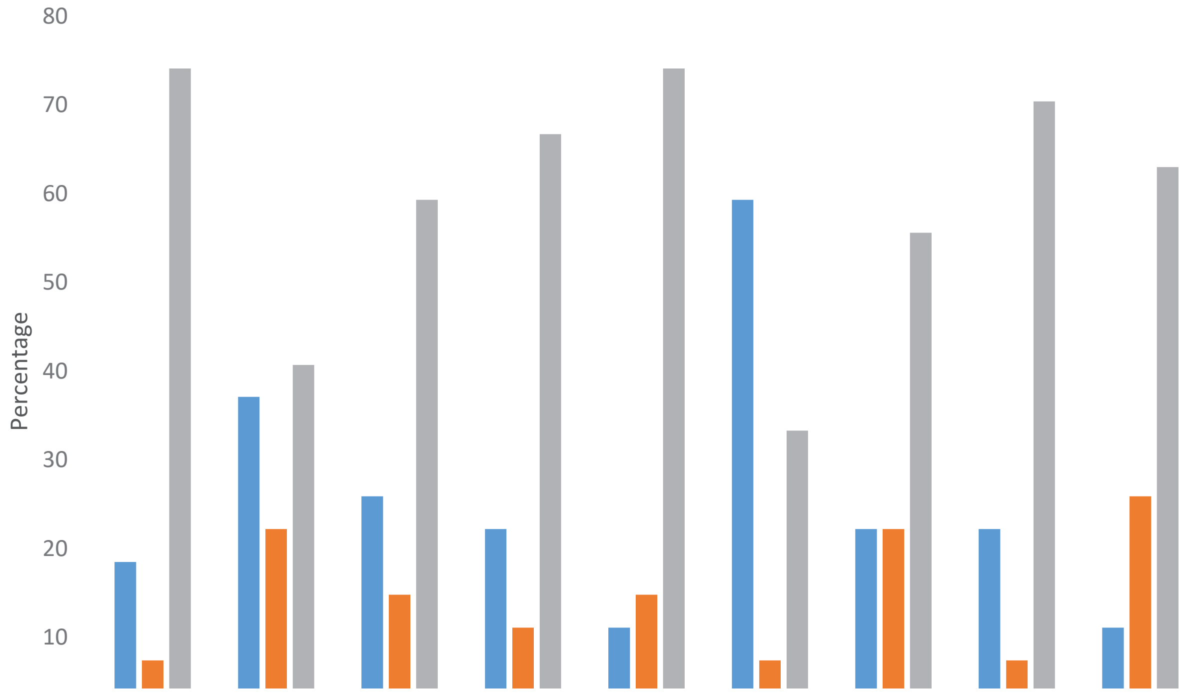

Figure 2: Overall antibiotic susceptibility pattern of *Salmonella* isolated from meats sold in Lagos, Nigeria.
CIP: Ciprofloxacin; CHL: Chloramphenicol; TET: Tetracycline; CFX: Ceftriaxone; GEN: Gentamicin; AMO: Amoxicillin; AUG: Augmentin; AZI: Azithromycin and TRI: Trimethoprim

Multiple Antibiotic Resistance index (MAR) index of *E. coli*

The MAR of *E. coli* ranged between 0 to 0.89. Two *E. coli* isolated from beef and RTE mutton were susceptible to all the antibiotics used. 9 out of the 33 *E. coli* were resistant to 3 antibiotics which is the most frequent MAR recorded. The highest MAR recorded is 0.89 by *E. coli* isolated from RTE chicken Table 5.

Multiple Antibiotic Resistance index (MAR) index of *Salmonella*

The MAR index recorded in this study for *Salmonella* ranged between 0.11 to 0.67. The highest percentage (11/27) of *Salmonella* subjected to the antibiotic test exhibited resistance to two antibiotics. One isolate each showed resistance to 4, 5, and 6 of the antibiotics. Six *Salmonella* showed resistance to 1 antibiotic while seven were resistant to three antibiotics used in this study Table 6.

Discussion

The meats were expected to be free of bacteria and the highest population of coliform was recorded in beef. All the meats used in this study were positive for coliform contamination which is close to the value reported by Maharjan, et al. [19] who concluded that more than 80% of the meats collected were positive for coliform contamination. Coliform contamination of 3.52×10^7 cfu/g for chevon and 2.14×10^7 cfu/g for beef was reported by Antwi-Agyei and Maalekuu [20] which were higher than the level reported in this study. A lower coliform count of 3.12 logcfu/ml was recorded by Sowunmi, et al. in a study on raw meats obtained from Sabo Market in Ikorodu, Lagos State [21]. Kim and Yim reported an average coliform count of 0.37logcfu/g which is lower than the range documented in this study on the raw and RTE meats [22].

The phenotypic appearance of *E. coli* and *Salmonella* colonies on their respective selective media, Gram's reaction, and cultural biochemical tests profile were similar to the features reported by Feng, et al. [15]; and Raji, et al. [23]. These pathogens are known to reside in the gastrointestinal tract of humans and other animals, live close to one another, and circulate throughout the ecosystem through defecation, death of the harboring animal, and improper disposal of the waste. Bacteria in this close association tend to share and transfer traits like resistant genes among one another [24]. These pathogens are also known to get to the environment during slaughtering and get transferred to humans and other animals through contaminated feed, food, water, utensils, and uncooked and improperly cooked meats. The contamination of the meat and utensils used during the slaughtering of animals and animal processing has led to the transfer of these pathogens to humans and caused various enteric diseases that sometimes result in the death of humans in the absence of adequate medical attention. The consumption of meats and meat products contaminated with bacterial foodborne pathogens can lead to foodborne infections and outbreaks which are of great concern to the public health and public health experts. The contamination of these products with bacteria will result in short shelf life or early spoilage and unavoidable financial loss to farmers, sellers, and consumers [4].

Escherichia coli and *Salmonella* are some of the most important bacterial pathogens of humans and animals that

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can be transferred to meats and meat products and cause various foodborne infections, sickness, and death. The detection of *E. coli* and *Salmonella* in meat samples used in this study indicates the presence of lapses in the process of slaughtering the animals, handling, transportation, cooking, and display at markets [25]. The high prevalence of these bacteria could also be attributed to a lack of proper hygiene during the slaughtering of the animal as there are possibilities of the intestinal content ruptures to come in contact with the meats, surfaces, and utensils used during evisceration. Also observed during sampling was the proximity location of the waste dumping site to the preparation point contributing to the contamination as flies were seen settling on the meats. Another possible means of cross-contamination observed during sampling was the knives used for evisceration were also used to cut the meat into pieces. In the market, the meats are always on display in the open air without covering which brings potential pathogen-laden houseflies in contact with the meats. During the purchase of meats (beef, mutton) in the market, it is a common practice of potential buyers to inspect many meat portions with bare hands before a choice is made. Among the samples is a local meat preparation sold in Nigeria as 'suya'. The analysis of the sample yielded the least coliform population among all the samples analyzed despite the meat being exposed to naked fire. Analysis of the suya yielded no *E. coli* and *Salmonella* growth in this study.

Table 5: Antibiotic resistance profile and multiple antibiotic resistance index (MAR) of *E. coli* isolates isolated from Meat samples in Lagos, Nigeria.

E. coli code	Source	Antibiotic resistant profile	Number of antibiotics	MAR index
RFM2	RTE Mutton	Nil	0	0
KB2	Beef	Nil	0	0
AC1	Chicken	Tr	1	0.11
BF2	Guinea fowl	Au	1	0.11
SF3	Guinea fowl	Au	1	0.11
RHB1	RTE Beef	Au	1	0.11
RGB2	RTE Beef	CiCh	2	0.22
BX2	Chevon	AuAm	2	0.22
RGF2	RTE Guinea fowl	AuTr	2	0.22
AF1	Guinea fowl	TrAm	2	0.22
OF4	Guinea fowl	AmTe	2	0.22
RMX1	RTE chevon	AuTr	2	0.22
KF5	Guinea fowl	AuAm	2	0.22
KX4	Chevon	AuAmCh	3	0.33
SX1	Chevon	CiAuAm	3	0.33
OM1	Mutton	AuTeCe	3	0.33
BM2	Mutton	AmTeTr	3	0.33
SM4	Mutton	AuAmCh	3	0.33
AB4	Beef	AuTrAz	3	0.33
RMM3	RTE Mutton	AmTeCe	3	0.33
RHF3	RTE Guinea fowl	AmChTe	3	0.33
RGM4	RTE Mutton	AuAmCh	3	0.33
REM1	RTE Mutton	AuChCeTr	4	0.44
OB3	Beef	AuAmTrAz	4	0.44
RMF1	RTE Guinea fowl	AmChTrAu	4	0.44
SB1	Beef	AuAmChTeTr	5	0.56
OX3	Chevon	AuAmTrTeCe	5	0.56
RFX2	RTE chevon	AuAmChTrAz	5	0.56
RFC2	RTE Chicken	AuAmChTeCe	5	0.56
RGC3	RTE Chicken	CiChTeCeAmAu	6	0.67
BC3	Chicken	AuCeAzTrTeCh	6	0.67
OC4	Chicken	TeCeGeAmAuAzTr	7	0.78
REC1	RTE Chicken	ChTeCeGeAmAuAzTr	8	0.89

Ci: Ciprofloxacin; Ch: Chloramphenicol; Te: Tetracycline; Ce: Ceftriaxone; Ge: Gentamicin; Am: Amoxicillin; Au: Augmentin; Az: Azithromycin and Tr: Trimethoprim

Table 6: Antibiotic resistance profile and Multiple antibiotic resistance index (MAR) of *Salmonella* isolated from Meat samples in Lagos, Nigeria

E. coli code	Source	Antibiotic resistant	Number of antibiotics	MAR index
OF4	Guinea fowl	Te	1	0.11
KF5	Guinea fowl	Te	1	0.11
BM2	Mutton	Ce	1	0.11
RHX3	RTE Chevon	Am	1	0.11
RHM5	RTE Mutton	Ch	1	0.11
RGB2	RTE Beef	Ce	1	0.11
SX1	Chevon	TeGe	2	0.22
RFF5	RTE Guinea fowl	GeAm	2	0.22
OX3	Chevon	GeAm	2	0.22
KM5	Mutton	CiCe	2	0.22
SB1	Beef	ChCe	2	0.22
AF1	Guinea fowl	TeAm	2	0.22
BF2	Guinea fowl	GeAm	2	0.22
RFX2	RTE Chevon	AmAu	2	0.22
REB4	RTE Beef	ChTr	2	0.22
REF4	RTE Guinea fowl	ChAz	2	0.22
KB2	Beef	ChAm	2	0.22
SC2	Chicken	ChAuAm	3	0.33
AB4	Beef	ChAmAu	3	0.33
AX5	Chevon	AmAuTr	3	0.33
OC4	Chicken	CiTeAm	3	0.33
RMC5	RTE Chicken	ChGeAz	3	0.33
REC1	RTE Chicken	ChAmAz	3	0.33
RGM4	RTE Mutton	CiAmAz	3	0.33
BC3	Chicken	CiTeAmAu	4	0.44
AC1	Chicken	AmAuAzTrCe	5	0.56
RFC2	RTE Chicken	ChTeCeAmAZCi	6	0.67

Ci: ciprofloxacin; Ch: chloramphenicol; Te: tetracycline; Ce: Ceftriaxone; Ge: Gentamicin; Am: Amoxicillin; Au: Augmentin; Az: Azithromycin and Tr: Trimethoprim

In this study, *E. coli* was absent in 14 (28%) meats while Salmonella was absent in 16 meats (32%). This high prevalence clearly shows the danger associated with meat consumption. A

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similar level of *E. coli* contamination in retail chicken (83.5%) and beef (68.9%) was reported by Zhao, et al. [26] in the US. Kim and Yim [22] conducted a study on various livestock meats sold in the Korean Republic, *E. coli* was found to be absent, but coliforms were detected. The absence of these pathogens is the reflection of adherence to the practices suggested by Adzitey at the time of the study [27]. The situation is subject to changes as there is a possibility of opposite results in future studies that may result from management changes, non-adherence to the quality management system, and poor hygiene. A lower value of *E. coli* contamination was reported by Adzitey [27]; and Altalhi, et al. [28] on various meat samples studied in comparison with this study. In a study conducted by EFSA [29] on meat products and meat preparations from mixed sources, low levels of contamination of 2.7% by non-0157 *E. coli* and 2.2% by Shiga toxin-producing *E. coli* were reported. Ciekure, et al. [30] studied ready-to-eat meat (RTE) collected in Lavia, it was discovered that 32% of the meat products were contaminated with *Escherichia coli* despite being exposed to heat. Also, 54% level of *E. coli* contamination was detected in raw beef in a study by Adzitey [27] and 76% in a study by Parvin, et al. [31] on frozen chicken meat. Adeyanju and Ishola [32] obtained a lower prevalence of 33% for *Salmonella* and 43.4% for *Escherichia coli* isolated from retailed poultry meat and processed meats sold in Oyo State, Nigeria. In a comparative study to determine the level of *E. coli* contamination between locally made chicken meat and chicken thigh imported into Ghana, the study showed higher levels of *E. coli* contamination on local chicken (64.29%) than on imported chicken (55.30%) Rasmussen, et al. [33]. Another study on the prevalence of *E. coli* O157:H7 from beef in Kwara State Nigeria, showed a 5.6% prevalence which is lower than the 80% recorded in this study [34]. A higher prevalence (56%) of *E. coli* than *Salmonella* (31%) was reported by Adzitey, et al. which agrees with our study's result [35]. A similar study on *E. coli* obtained from meats in Sabo, Ikorodu region of Lagos state showed 82% overall prevalence (higher than this study prevalence by 10%) with raw sheep and raw Guinea fowl (87.5%) each, raw beef (85%), raw local chicken (77.5%) and raw goat (72.5%) [21].

In this study, a higher percentage of contamination was discovered in raw meats than in ready-to-eat meats (RTE). The lower percentage of these bacteria in RTE is attributed to the process of cooking, frying, and drying that exposed the meat to high temperatures and killed the bacteria present in the meat together. It is expected that the level of contamination of meat in countries where proper adherence to handling and processing protocol of animals into meat to be lower than in developing countries with low regard to proper handling and hygiene practices. The process of preventing and controlling bacterial foodborne pathogens begins with proper handling and hygiene from slaughtering houses to the last link of consumption at various homes and offices. Minimizing the presence of bacteria including coliforms, fecal coliforms, *Salmonella*, and other pathogenic bacteria is vital in ensuring the quality health of the populace. The control and preventive measures described by Adzitey [36] must be adhered to in order to prevent foodborne infections. Good agricultural practices during activities such as animal breeding, rearing, slaughtering, transporting animals, etc. are the first line of action to prevent foodborne diseases caused by bacteria Adzitey [37]. Regular and proper waste disposal, and avoidance of contact between waste, equipment, food, and water have also been in practice to avoid contamination. Great emphasis should be placed on thorough hygienic handling and processing of animals into meat and meat products to reduce the level of morbidity and mortality caused by *E. coli*, *Salmonella*, and other pathogens.

The high level of resistance to commercial antibiotics in this study is a great concern as these bacteria have acquired different mechanisms to overcome the antibacterial effect. This high level of resistance recorded in this study and past studies revealed the high level of risks associated with the overuse and the abuse of antibiotics in animal husbandry and these bacteria eventually make their way into the environment and human population and continue circulation between animals and humans. The consumption of these meats poses a great danger as the antibiotic-resistant bacteria are transferred to humans. It also leads to the accumulation of antibiotic residue and the spread of these antibiotic-resistant genes within the hosts which will result in drug failure when treating diseases caused by these pathogens in the future. In this study, only two *E. coli* isolated from beef and RTE mutton were susceptible to all the antibiotics used without any *salmonella* isolated from RTE meat being susceptible to azithromycin. Gentamicin was the most potent antibiotic as it showed susceptibility of 84.8 and 74.1% against *E. coli* and *Salmonella* respectively. Zhao, et al. [26] reported higher levels of resistance (18.6%) by *E. coli* isolated from different retail meats in the USA to gentamicin. A higher susceptibility to ciprofloxacin (95.56%) and trimethoprim (82.22%) but a lower susceptibility to gentamicin (75.56%) was reported on *E. coli* isolated from raw meats in Ghana [38]. Adzitey, et al. [39] found the highest level of resistant *E. coli* in the hands of meat sellers which is similar to the high MAR index reported in the RTE meat reported in this study. In a study by Hassan, et al. [40], a total of 34 bacteria were isolated from raw chicken of which 97%, 94%, 64%, 50%, 32%, 24%, 21%, and 6% were resistant to oxacillin, penicillin, ampicillin, cefotaxime, tetracycline, erythromycin, ciprofloxacin, and gentamicin respectively. *E. coli* and *Salmonella* were also identified as one of the most prevalent bacteria with the highest resistance. Also, a study conducted on meat and its products in Kalobia, Egypt by Abd-El-Tawab, et al. [41] showed that 11 (6.3%) of the bacteria isolated were *E. coli* and were highly resistant to methicillin and oxacillin but sensitive to enrofloxacin and gentamicin. The finding of this study on the sensitivity of *E. coli* to gentamicin is similar to the result obtained by Hassan, et al. [40] and this present study. In a similar study by Sowunmi, et al. in Lagos State the MAR of *E. coli* isolated from raw meats ranged between 0-88 which agrees with MAR recorded on *E. coli* in this study [21]. Also, most of the *E. coli* from the study had MAR of 0.38 which is also close to 0.33 recorded for most *E. coli* in our study. Higher susceptibility values were recorded for gentamicin (88.3%), trimethoprim (85%), chloramphenicol (83.3%), and ceftriaxone (80%) were reported [21]. In another study by Adetunji and Ishola [42], the virulent strains of *Salmonella* and *E. coli* were found to have 60% resistance each while the non-virulent strains had a lower

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resistance of 50% each. Also, a study by Adzitey, et al. [43] in which 84.0, 88.9, 88.0, 89.0. 86.7, 80.0 and 75.6% prevalence of *E. coli* was reported in meat, mutton, guinea fowl, beef, local chicken, and chevon respectively with 85, 73.33 and 71.69% of *E. coli* being resistant to erythromycin, tetracycline, and ampicillin respectively while 68.33% (41/60) of the identified *E. coli* exhibited resistance to two or more antibiotics. Pławinska-Czarnak, et al. studied *Salmonella* obtained from raw meats in Poland and obtained 53.84% of several *Salmonella* strains to be multidrug resistant [44]. The MAR index of the study on *Salmonella* ranged between 0.09 -0.51 which falls in the MAR range recorded in our study [44]. A contrary report on *E. coli* was reported by Saud, et al. that tetracycline had resistance values of 60.6% while the resistance value reported for gentamicin agrees with this study result [45]. Another contrary result on *E. coli* isolated from raw meats showed 75% resistance to tetracycline [46].

The resistance to the 3rd generation cephalosporins; ceftriaxone by *E. coli* and *Salmonella* in this study poses a serious concern due to the extensive use of ceftriaxone in the treatment of diarrhea and Salmonellosis in humans. A similar report on *Salmonella* isolated from raw meats to be resistant to the third-generation cephalosporins family was documented by Plawinska-Czarnak, et al. [44]. This spread of resistance to 3rd generation cephalosporins and beta-lactam groups which are in high use in human medicine is worrying. AmpC and extended-spectrum beta-lactamase are usually antibiotic-resistant mediated genes in *E. coli* and *Salmonella*. 36.4% and 25.9% of *E. coli* and *Salmonella* were resistant to tetracycline despite the embargo imposed on its non-therapeutic use in animal treatment. Augmentin: an antibiotic that contains amoxicillin (beta-lactam family) and clavulanic acid (beta-lactamase inhibitor) showed greater antibacterial activity on both *E. coli* and *Salmonella* than amoxicillin. This higher activity can be attributed to the presence of clavulanic acid in Augmentin which created synergistic antibacterial action with amoxicillin on these pathogens. The high level of *E. coli* and *Salmonella* resistance to amoxicillin may be attributed to being the commonest and cheapest antibiotics in Nigeria's locality which makes its use on a higher level than other antibiotics. It is widely used for the treatment of diarrhea in animal husbandry and human medicine, and this could have led to the acquisition and development of survival strategies.

The MAR index of *E. coli* ranged from 0-0.89 antibiotics while *Salmonella* ranged from 0-0.69 antibiotics. 2 *E. coli* isolates were found susceptible while none was found resistant to all the antibiotics used in this study. 4, 7, 9, and 3 *E. coli* showed MAR of 11%, 22%, 33%, and 44% while 4, 2, 1, and 1 *E. coli* had MAR indexes of 56%, 67%, 78%, and 89% respectively. None of the *Salmonella* was found susceptible and resistant to all the antibiotics used in this study. 6, 11, 7, 1, 1, and 1 *Salmonella* had MAR of 11%, 22%, 33%, 44%, 56%, and 67% respectively. Kathleen, et al. [18] reported that bacteria with a MAR index above 2 were from an environment with high antibiotic usage and bacteria with a MAR below 2 were from an environment with low utilization of antibiotics for disease treatment and prevention. The result of the antibiotic susceptibility of *E. coli* and *Salmonella* obtained from meats in this study can be categorized as multidrug-resistant as a high percentage of the bacteria exhibited resistance to more than two antibiotics. In this study, 61% of the *E. coli* had a MAR index between 0.33-0.88 which indicates that these bacteria are from environments where antibiotics are largely used, misused, and abused. A lower trend was observed in *Salmonella* of which 37% had a MAR range of 0.33-0.67. *E. coli* and *Salmonella* isolated from RTE meats possessed a higher MAR index with the highest source of contamination being attributed to human contact after cooking, frying, smoking, oven drying, etc. Humans are thereby harboring many of these multi-drug resistant *Salmonella* and *E. coli* which could cause failure in treating Salmonellosis and *E. coli* infections in the future. Adzitey, et al. [47] reported 41 (68.33%) of the *E. coli* isolated from livestock sold in Ghana metropolis to be multi-drug resistant to three or more antibiotics. Saud, et al. [45-36] reported 52.5% multi-drug resistance by *E. coli* isolated from meats and 69.81% multi-drug resistance by *E. coli* isolated from various samples used in the study. Altalhi, et al. [28] found that 86.5% of E. *coli* were resistant to at least one antibiotic and 40.5% were resistant to at least three antimicrobials. 68.33% MDR was reported for *E. coli* which is closer to the value reported in this study (61%) [21]. Slaughtering of animals only after the withdrawal days of antibiotic treatment is a way out as it will reduce the spread of antibiotic residue in the animals to humans and antibiotic-resistant bacteria. Ekli, et al. reported that 73.2% of farmers in Wa municipality of Ghana did not practice the withdrawal method before animals were slaughtered for consumption [48]. The consumption of animals killed before the withdrawal day of antibiotic treatment will lead to the accumulation of these antimicrobial residues in the body which the intestinal flora will become adapted to. This will possibly lead to later time failure of such antibiotics in disease treatment.

The data obtained in this study poses serious challenges to one health concept as *E. coli* and *Salmonella* were found to be present in meats meant for public consumption. These bacteria were also found to be multidrug resistant. The antibiotic-resistant genes contained in *E. coli* and *Salmonella* can be transmitted to the gut microbiota of humans and create more antibiotic-resistant strains in humans. These antibiotic-resistant bacteria will later be released into the environment through defecation which will lead to the continuous spread of antibiotic-resistant bacteria and the exchange of antibiotic-resistant genes in the environment. This cycle continues again through the ingestion of contaminated forage, water, and soil by animals which are then consumed by humans and then to the environment. The health of the environment is critical in maintaining the health and well-being of plants, humans, and animals. Unsafe water, poor sanitation, and poor hygiene are responsible for human and animal mortality and morbidity, particularly in vulnerable populations in low-income countries.

Nonetheless, these results must be interpreted with caution and limitations should be borne in mind. The findings of this study are time-based as results from future studies may show deviation due to hygiene practices and adherence to the withdrawal method. Also, this study does not give the trueness

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of the *E. coli* and *Salmonella* prevalence in Lagos state as few cities were considered for the study, hence, future studies are encouraged to focus on the state at large and higher number of samples. In addition, there is little or lack of data available or prior research on similar studies in the state, thereby the need for future research to give more insight into these pathogens' prevalence and susceptibility to antibiotics. The virulence factors and genes responsible for the resistance in *E. coli* and *Salmonella* were not investigated due to the limitations of the available facilities in the laboratory. Further studies are encouraged for the determination of genes responsible for the resistance to these antibiotics, the mechanisms used to transfer these genes, and the possibility of drug combination as seen in Augmentin. Also, future studies should be directed towards determining the possibility of cross-resistance between these *Salmonella* and *E. coli* within the host.

Conclusion

Overall, 50 (100%) of the meat samples were positive for coliform with 72 and 68% positive for *E. coli* and *Salmonella* respectively. Raw meats were contaminated more than RTE meats which shows a reduction in the contamination level due to the exposure to heat during processing. However, adequate hygiene practices are recommended for RTE meat sellers to prevent the spread of pathogenic bacteria among humans, animals, and the environment. The phenotypic antibiotic resistance revealed *E. coli* to be highly resistant to Augmentin, amoxicillin, and trimethoprim, and high susceptibility to ciprofloxacin, gentamicin, and azithromycin. Also, *Salmonella* showed high levels of resistance to amoxicillin and high susceptibility to all the antibiotics except amoxicillin and mild to chloramphenicol. To overcome the problem of high resistance to antibiotics observed in this study, there is a need for farmers to practice the withdrawal method, reduce the level of antibiotic use, and practice proper hygiene and management practices. This is to reduce the use of antibiotics in treating diseases and the spread of antibiotic-resistant bacteria among animals and the environment. An organic system of preventing and treating diseases should be adopted among the farmers. It is also recommended that farmers, butchers, meat sellers, and the public be given proper awareness regarding good hygiene practices. The government authority from the local to the federal level should establish a body that will exercise regular meat inspection before killing and distribution to the public.

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