Bacterial Load and Antimicrobial Profile of *Escherichia coli* and *Listeria spp*. Isolates from Muscle Tissues of Slaughtered Cattle at a Major Abattoir in Ibadan, South-Western Nigeria

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Abstract: Meat is prone to contamination by pathogenic organisms during slaughter and processing due to unhygienic practices in Nigeria. In recent times, there has been an increase in the prevalence of antibiotic resistant foodborne pathogens due to increased drug misuse in livestock industry. We assessed the level of microbial contamination of fresh muscle tissues from cattle slaughtered in a major abattoir in Ibadan immediately after slaughter and also determined the antibiogram of *Escherichia coli* and *Listeria* spp isolates from the muscle tissues. These were done using standard plate and Bauer-Kirby disc diffusion techniques for bacteriological assay and antibiotic sensitivity testing, respectively. We found that the muscle tissues from the slaughtered cattle were highly contaminated, with the Total Aerobic (6.59±0.94 log cfu/g), coliform (6.43±0.67 log cfu/g) and *Listeria* (6.96±0.32 log cfu/g) counts being higher than the acceptable international standards. Isolated *E. coli* and *Listeria* spp demonstrated 100% resistance to all tested antibiotics. We thus recommend further studies to be carried out on the molecular characteristics of antibiotic resistant genes responsible for transferability of bacterial resistance among foodborne pathogens in Nigeria.

Keywords: Beef, microbial contamination, *E. coli*, *Listeria* spp, antibiotic resistance.

INTRODUCTION

Microbial food contamination and resistance of foodborne pathogens to commonly used antibiotics have been a continual problem in food industries across the globe. While food protection from microbial hazards has received substantial concerns from stakeholders in food safety and the public in the developed countries, the same cannot be said of the developing countries. Worse still, several foodborne disease outbreaks are being underreported in most developing countries especially in the sub-Saharan Africa. Cattle serve as reservoir host to pathogens especially *Escherichia coli* which makes beef to be prone to contamination by pathogenic organisms during slaughter and processing particularly due to unhygienic practices which characterize most developing countries. *Listeria* spp (especially *Listeria monocytogenes*) could also contaminate meats facilitating the transmission of listeriosis among susceptible individuals.

Foodborne antimicrobial resistance is a biological hazard [1]. Bacterial resistance to multiple antimicrobials became a health problem in the 1980s [2]. Resistant *Escherichia coli* strains can easily colonize humans after consumption of contaminated beef [1]. Resistance of pathogens to multiple antibiotics encourages pathogen persistence in food processing environment, prolongs treatment of disease conditions and increases hospitalization rates and mortality in animals and humans [1, 3, 4].

Over the years, reports have shown that resistance of foodborne pathogens is on the increase [5, 6]. This is mainly due to increase and misuse of antibiotics in food animals [4, 7, 8]. In most Nigerian cattle markets, treating cattle with antibiotics is a common practice despite the fact that these cattle are for slaughter within few days. Consequently, antibiotic residues have been detected in ready-to-eat meats in Nigeria [7, 9, 10].

Though most researchers have assayed the bacteriological quality of meat tables and other processing facilities in Nigerian abattoirs [11, 12]; there are limited studies determining the microbial quality of cattle tissues sold for public consumption. Whereas, beef constitutes a major animal protein source consumed in Nigeria. Since antimicrobial resistance of foodborne pathogens has been recognized as a public health hazard, there is need to investigate the antibiotic sensitivity profiles of meat-borne pathogens in the country. Therefore, we assessed the level of microbial contamination of fresh beef immediately after slaughter and also determined the antibiogram of *Escherichia coli* and *Listeria* spp isolates from the tissues. This will provide current baseline information on bacteriological quality, resistance patterns of *Escherichia coli* and *Listeria* isolates and associated health risks from beef processed and sold for human consumption in Ibadan, South-Western Nigeria.

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MATERIALS AND METHODS

Study Site, Animal Sampling and Tissue Collection

The sampling site was the Bodija Municipal Abattoir, a major slaughterhouse in South-Western Nigeria. Averagely, 250 cattle are slaughtered daily in this abattoir and it supplies bulk of the meat consumed in Ibadan (with over 1.3 million population: [13]) and its surrounding environment. One in every ten cattle slaughtered was randomly sampled with a total of ten cattle sampled per day. Overall, a total of 30 animals were sampled over a three day visit to the abattoir. Muscles tissues of approximately 50 g each were obtained in 3 replicates from the 30 animals resulting in 90 samples in all. They were collected aseptically into sterile sample bags and transported in an insulated flask packed with ice to the laboratory for analysis.

Bacteriological Assay

Microbial isolation was done according to Barrow and Feltham [14]. Fifty mL of 0.1% peptone water (Lab M, UK) was added to each stomacher bag containing 50 g of the muscle tissue. This was then macerated to ensure proper mixture of samples. Serial 8-fold dilutions (10^{-8}) of the homogenates were made in peptone water and plated by standard pour plate method. Appropriate dilutions were surface plated on Plate count agar (Lab M, UK) for Total Aerobic Plate Count (TAPC), MacConkey agar (Lab M, UK) for Coliformcount (CC) and *Listeria* selective agar (Fluka, Germany) for *Listeria* count. *E. coli* and *Listeria* spp were further isolated and identified. All media were prepared as directed by the manufacturers. Plates were incubated at 37ºC for 18-24 h. This was done in triplicates. Counts were expressed as log cfu per gram in mean ± standard deviation.

Antibiotics Sensitivity Test

Antibiogram of *E. coli* and *Listeria* spp was carried out using the Bauer-Kirby disc diffusion method [15]. Briefly, 1 mL × 10^{-8}cfu of a 24 h nutrient broth culture of *E. coli* and *Listeria* spp isolates obtained were inoculated into freshly prepared nutrient agar (Lab M, UK) plates in replicates. These inoculated plates were left to dry before sensitivity discs were applied and then incubated for 24 h at 37^0C. Zones of inhibition, indicative of sensitivity of the isolates to the tested antibiotics, were measured. Gram –ve and +ve discs (Abtek Biological Ltd, England) were used for the *E. coli* and *Listeria*spp, respectively. The gram –ve disc contained the following antibiotics: Tetracycline (TET) (10µg), Augmentin (AUG) (30µg), Ofloxacin (OFL) (30µg), Gentamycin (GEN) (10µg), Nalixidic acid (NAL) (30µg), Nitrofurantoin (NIT) (20µg), Cotrimoxazole (COT) (25µg) and Amoxicillin (AMX) (30µg). Cotrimoxazole (COT) (25 µg), Chloramphenicol(CHL) (10 µg), Cloxacilline (CLO) (5 µg), Erythromycin (ERY) (5 µg), Gentamicin (GEN) (10 µg), Augmentin (AUG) (30 µg) and Streptomycin (STR) (10 µg) were the antibiotics on the gram +ve disc.

Statistical Analysis

Using the SPSS version 15 a One-way ANOVA was used to determine significance in mean bacterial counts in the muscle tissues and sensitivity of isolates to groups of antibiotics. Zones of inhibition were computed into mean ± standard deviation in mm. All analysis was carried out at $P<0.05$.

RESULTS AND DISCUSSION

Microbial counts (log cfu/g) obtained from the slaughtered cattle muscle tissues is presented in Table **1**. Generally, very high bacterial loads were observed. Total Aerobic, coliform and *Listeria* counts ranged from 5.45±0.21 -8.12±0.05 log cfu/g, 0.0±0.0 - 8.21±0.02 log cfu/g and 0.0±0.0 - 7.65±0.01 log cfu/g, respectively. Averagely, these counts were significantly different ($p < 0.05$) from one another with the *Listeria* count being the highest (6.96±0.32 log cfu/g). The results of this study show that tissues from slaughtered cattle at the Ibadan abattoir were highly contaminated, with the Total Aerobic, coliform and *Listeria* counts being higher than acceptable international standards (New Zealand Food Safety Authority, [16], Commission of the European Communities, 2005 [17]). In addition, the *E. coli* and *Listeria* spp isolates showed 100% resistance to all tested antibiotics. This microbial contamination detected in this study is comparable to another study on microbial load in which samples were sourced from the same study site and surrounding markets where lower counts on meat tables and meat table scrapings were reported. Both the coliform and *Listeria* counts increased with about two logs after meat tables were sampled after sales [11], though the counts obtained were higher than those reported in this study. This indicates that microbial loads on beef tissues increase even after slaughter and processing. This is a clear indication that meat processing and handling after slaughter is unhygienic. Though Fasanmi *et al*. [12] found lower bacterial counts (2.78 × 10^5cfu/ml) on meat display tables, higher total plate (26.50 ± 16.7 - 318.00

Table 1: Microbial Load (log cfu/g) on Muscles Tissues of Slaughtered Cattle at the Ibadan Municipal Abattoir

Sample	Total Aerobic plate count	Coliform count	*Listeria* count
	log cfu/g		
1	5.80±0.45	5.76±0.40	6.69±0.13
2	5.56±0.79	5.78±0.00	7.05±0.01
3	5.45±0.21	6.22±0.11	6.98±0.11
4	6.18±0.04	6.25±0.10	6.96±0.18
5	5.66±0.26	6.29±0.12	6.85±0.03
6	6.02±0.34	6.24±0.18	6.82±0.06
7	7.46±0.21	6.98±0.03	7.03±0.01
8	5.83±0.18	6.08±0.43	6.64±0.07
9	5.66±0.26	5.77±0.10	6.95±0.07
10	5.45±0.21	5.84±0.09	6.91±0.06
11	5.90±0.08	5.35±0.49	6.89±0.67
12	5.48±0.67	6.40±0.28	6.95±0.10
13	5.60±0.43	0.00±0.00	0.00±0.00
14	5.95±0.07	6.28±0.03	6.84±0.16
15	5.94±0.14	6.10±0.28	7.22±0.01
16	7.84±0.06	0.00±0.00	6.55±0.07
17	7.09±0.02	5.90±0.00	6.66±0.35
18	6.62±0.06	6.78±0.00	6.45±0.04
19	7.48±0.24	5.78±0.00	7.21±0.01
20	8.12±0.05	7.12±0.01	7.08±0.10
21	7.51±0.00	8.21±0.02	6.65±0.92
22	7.94±0.14	7.31±0.01	6.96±0.00
23	7.40±0.03	6.15±0.00	6.78±0.26
24	6.70±0.45	6.82±0.00	7.28±0.00
25	7.62±0.01	7.25±0.02	7.41±0.15
26	7.70±0.45	7.23±0.01	7.65±0.01
27	6.07±0.16	6.20±0.08	7.00±0.03
28	6.22±0.63	6.58±0.62	7.05±0.24
29	7.62±0.15	6.60±1.16	7.36±0.04
30	7.79±0.24	7.31±0.00	7.03±0.00
Total	6.59±0.94[b]	6.43±0.67[a]	6.96±0.32[c]

[a,b,c]Means with same superscripts are not significantly different at 0.05 level across the sample types and across the strains type*International standard: Aerobic Plate Count=6.7log, coliforms=3.0log, Listeria=not detectable in 25gm.

± 58.7 × 10^6 cfu) and coliform (18.25 ± 7.8 - 338.50 ± 57.5 × 10^3cfu) counts were obtained in both public and private abattoirs elsewhere in Southern Nigeria [18]. These higher counts could have resulted from the unhygienic handling practices of abattoir butchers and other abattoir workers. As reported, contamination usually arises from unwholesome contacts of meat with excretions from skin, mouth and nose of the abattoir workers [18, 19].

Our result is also comparable to the aerobic plate and coliform counts obtained in some beef samples in other developing countries. In Cote d'ivoire, Koffi-Nervy *et al*. [20] recorded a range of 4.93-8.1 log cfu/g and 1.83-4.73 log cfu/g, respectively for total plate and feacal coliform counts. Also, a mean range of 1.20 × 10^6 - 7.57× 10^6cfu/cm^2 and 5.57 – 6.22 log cfu/cm^2 for total bacterial counts in some retail outlets and abattoirs were recorded in northern Ghana [21, 22].

Averagely, TAPC was not more than 9 log in beef and processing environments in Pakistan [23, 24]. Mean AP and Total coliform counts were 1.62×10^5cfu/g and 5.29×10^1cfu/g respectively in an abattoir in Ethiopia [25] while Haileselassie *et al*. [26] recorded the bacterial loads in meat from abattoir, butchers shop and street meat sale as 1.1×10^5cfu/g, 5.6×10^5cfu/g and 4.6×10^6cfu/g, respectively in Mekelle city. Meat contamination with coliforms indicates poor hygienic conditions of carcass processing [27]. This means maintenance of a high level of hygiene and sanitation in meat processing facilities will reduce microbial contamination.

Furthermore, Adetunji and Isola [11] reported higher *Listeria* counts of 8.20 ± 0.06 log cfu/cm^2 and 10.47 ± 0.05 log cfu/cm^2 for meat display tables before and after sales in Ibadan. This indicates the presence of contaminating agents in every step of slaughtering and processing of beef for sale. However, *Listeria* counts from beef samples reported in an abattoir in Southeast Nigeria were not greater than 5.90 log cfu/g [28]. The findings in this present study where significantly ($p<0.05$) higher than international limits thereby incriminating that beef could be a source of listeriosis and other infections. Abattoir operations in developing countries, particularly Nigeria lack quality control despite the fact that various microbial decontamination strategies have been identified [29].

The *E. coli* isolates showed some level of sensitivity to six of the antibiotics tested (Table **2**) though were resistant to all antibiotic. AMX and Aug were excluded from the analysis because they generally had zero (mm) zones of inhibition. Averagely, the highest sensitivity (2.50±0.60mm) level was observed with ofloxacin which was significantly different ($p < 0.05$) from other antibiotics except gentamycin. Other tested antibiotics which produced above 1.00mm zones of inhibition with the *E. coli* isolates were TET, NAL and NIT. The resistance pattern common to all the isolates was: AMX, AUG, COT, GEN, NAL, NIT, OFL, TET. Though the *E. coli* isolates produced zones of inhibition with some of the antibiotics, this is still comparable with other findings. Adetunji and Isola [30] showed that *E. coli* (abattoir isolates) were sensitive to cefuroxime, ciprofloxacin and gentamicin and norfloxacin, but less sensitive to tetracycline, amoxillin, gentamicin and chloramphenicol. In another report, Olatoye [31] recorded the resistance of pathogenic *E. coli* to four or more antibiotics while poultry isolates of *E. coli* were resistant to tetracycline, augmentin, cotrimozaxole and amoxicillin [32]. Similarly, it has been confirmed that *E. coli* is resistant toampicillin, streptomycin, tetracyclines and trimethoprim [1].

The 14 *Listeria* strains displayed varying low sensitivity levels to three of the seven antibiotics tested (Table **3**). All the strains were resistant to all antibiotics tested (100%). The *Listeria* strains were resistant (zones of inhibition = 0.00±0.00mm) to COT, CLO, ERY and AUG. The mean zone of inhibition observed with GEN was1.90±0.40mm which was significantly different from CHL (1.50±0.70mm) and STR (1.10±1.00mm). The resistance pattern noticed among all the *Listeria* isolates was for CHL, GEN, STR, COT, CLO, ERY and AUG. The complete resistance by *Listeria* spp. to the antibiotics observed in this study is higher than most findings by other researchers. These results confirm the presence of widespread resistant *E.*

Table 2: Resistance Profile (mm) Produced by *E. coli*

	Antibiotics					
Strain	**COT**	**GEN**	**NAL**	**NIT**	**OFL**	**TET**
MC02	0.00 ± 0.00^{R}	1.80 ± 0.40^{R}	1.80 ± 0.60^{R}	1.80 ± 0.30^{R}	2.50 ± 1.10^{R}	2.10 ± 0.80^{R}
MC03	1.50 ± 0.80^{R}	2.50 ± 0.70^{R}	1.30 ± 0.40^{R}	2.40 ± 0.90^{R}	2.60 ± 1.30^{R}	2.40 ± 0.50^{R}
MC08	1.80 ± 0.30^{R}	2.70 ± 1.10^{R}	1.90 ± 0.10^{R}	2.00 ± 0.70^{R}	2.60 ± 0.60^{R}	2.10 ± 0.60^{R}
MC09	0.00 ± 0.00^{R}	1.60 ± 0.10^{R}	2.10 ± 0.50^{R}	0.00 ± 0.00^{R}	2.20 ± 0.50^{R}	0.00 ± 0.00^{R}
MC10	0.00 ± 0.00^{R}	2.00 ± 0.60^{R}	2.00 ± 0.20^{R}	1.80 ± 0.30^{R}	2.40 ± 0.80^{R}	0.00 ± 0.00^{R}
MC14	1.90 ± 0.30^{R}	2.00 ± 0.04^{R}	1.90 ± 0.60^{R}	2.20 ± 0.60^{R}	2.70 ± 0.80^{R}	2.30 ± 0.60^{R}
MC17	0.00 ± 0.00^{R}	2.00 ± 0.07^{R}	2.00 ± 0.20^{R}	1.80 ± 0.30^{R}	2.40 ± 0.80^{R}	1.60 ± 0.20^{R}
MC19	1.80 ± 0.40^{R}	1.90 ± 0.30^{R}	2.00 ± 0.20^{R}	1.70 ± 0.40^{R}	2.60 ± 0.60^{R}	0.00 ± 0.00^{R}
Total	0.90 ± 0.90^{a}	2.00 ± 0.60^{cd}	1.80 ± 0.40^{bc}	1.70 ± 0.80^{bc}	2.50 ± 0.60^{d}	1.30 ± 1.10^{ab}

[a,b,c]Zones of inhibition with the same superscripts are not significantly different at 0.05 level.

Table 3: Resistance Profile (mm) Produced by *Listeria* spp.

	log cfu/g Antibiotics		
Strain	**CHL**	**GEN**	**STR**
LM01	1.90±0.10^{R}	2.00±0.30^{R}	1.90±0.50^{R}
LM02	2.20±0.20^{R}	1.70±0.30^{R}	0.00±0.00^{R}
LM03	1.90±0.30^{R}	1.80±0.40^{R}	2.20±0.50^{R}
LM04	1.70±0.40^{R}	1.80±0.40^{R}	0.00±0.00^{R}
LM04a	1.10±0.60^{R}	2.10±0.50^{R}	2.20±0.60^{R}
LM05	1.50±0.50^{R}	2.10±0.50^{R}	1.90±0.40^{R}
LM06	1.00±1.30^{R}	2.00±0.10^{R}	2.00±0.60^{R}
LM08	0.00±0.00^{R}	1.80±0.40^{R}	1.80±0.30^{R}
LM09	1.80±0.50^{R}	1.90±0.70^{R}	0.00±0.00^{R}
LM10	1.20±1.70^{R}	1.90±0.60^{R}	0.00±0.00^{R}
LM11	1.70±0.40^{R}	2.00±0.80^{R}	0.00±0.00^{R}
LM12	1.90±0.60^{R}	2.00±0.40^{R}	2.00±0.60^{R}
LM14	2.00±0.40^{R}	1.70±0.40^{R}	0.00±0.00^{R}
LM15	2.00±0.40^{R}	2.00±0.50^{R}	2.00±0.40^{R}
Total	1.50±0.70^{b}	1.90±0.40^{c}	1.10±1.00^{a}

^{a,b,c}Zones of inhibition with the same superscripts are not significantly different at 0.05 level.
R=resistant.

coli and *Listeria* spp. in the food processing environment. This is buttressed by the report of Adetunji and Isola [30] who found that *Listeria* spp isolated from the abattoir were resistant to tetracycline and highly sensitive to gentamicin, erythromycin and streptomycin. In another study, Adetunji and Odetokun [32] recorded a complete resistance of *Listeria* spp to cephalexin, clindamycin, augmentin, cotrimoxazole, ampicillin but were moderately sensitive to ciprofloxacin. Similar to our findings, Charpentier *et al*. [33], Yucel *et al*. [34] andIssa *et al*. [35] also reported 100% resistance of *Listeria* spp to antibiotics such as ampicillin and the cephalosporins. Despite the limitation of a small sample size in this present study, the resistance patterns obtained are suggestive of constant abuse of veterinary drugs in the food animal industry. For instance, tetracycline is often used as growth promoter and prophylaxis in the Nigerian livestock industry [31].

Since poor hygienic practices are not limited to the abattoirs in Nigeria alone, but also characterize most developing countries, we advocate that strict rules on hygiene and sanitation must be put in place at slaughter facilities. In addition, effective microbial decontamination strategies should be employed. Drug administration in the livestock industry should be restricted to veterinary personnel only and over-the-counter sale of antibiotics be banned. Besides, meat inspection strategies should be improved. Government should invest in a nation-wide surveillance to monitor microbial contamination and drug resistance trends in Nigeria while establishing standards for minimum and maximum microbial limits in meat sold for public consumption.

CONCLUSION

We found a high level of microbial contamination (bacterial loads greater than 6 log cfu/g) in muscle tissues from slaughtered cattle at a major abattoir in Ibadan, Nigeria which was higher than the acceptable international standards. Isolated *E. coli* and *Listeria* spp demonstrated 100% resistance to all tested antibiotics usually used for treatment and prophylaxis during cattle husbandary.

We recommend further studies to be carried out on the molecular characteristics of antibiotic resistant genes responsible for transferability of bacterial resistance.

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COMPETING INTEREST

We declare that there are no conflicting interests in the writing of this article.

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