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POSSIBLE TRANSMISSION OF DRUG-RESISTANT *SALMONELLA TYPHIMURIUM* AND *LISTERIA MONOCYTOGENES* WITHIN THE BEEF INDUSTRY IN ABUJA, NIGERIA

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ABSTRACT: Background: Widespread use of antibiotics in livestock production in large-scale across the globe has become of public and veterinary health importance because of its implication in antibiotic resistance. Adequate data in this area of research is not readily available in Nigeria; this study was undertaken in view of the possible link between antimicrobial resistance in farm animals and humans. **Methods:** We collected fifty samples of raw beef from different vendors and slaughter houses within Abuja and screened them for the presence of *Listeria monocytogenes* and *Salmonella typhimurium* using standard microbiological methods. The total bacterial and fungal counts, susceptibility of the isolates to different antibiotics and heat sensitivity at 55, 60 and 65ºC for 15 minutes were determined. **Results:** Our results show that ten isolates of *Listeria monocytogenes* and eighteen isolates of *Salmonella typhimurium* were isolated from the samples. The total viable bacteria count range was 1x 10^9 - 8x 10^9cfu/g while the fungal count was 1x 10^3 - 9x 10^9cfu/g. One (10 %) of the *Listeria monocytogenes* isolates was resistant to all antibiotics tested while all the *Listeria monocytogenes* isolates were resistant to cefuroxime. Eight (44.4%) of the *Salmonella typhimurium* isolates were resistant to at least three antibiotics. All the *Listeria monocytogenes* and *Salmonella typhimurium* isolates did not survive beyond 60 ^oC upon heat treatment. **Conclusions:** Our results indicate high prevalence of *Salmonella typhimurium* and *Listeria monocytogenes* in selected beef in Abuja. Beef therefore may represent a large reservoir for antimicrobial-resistant *Salmonella typhimurium* and *Listeria monocytogenes*.

INTRODUCTION: Beef is the flesh of a slaughtered cow, steer or bovine animal [1]. Although, these animals may be sold to be slaughtered as young as one to two years, finest quality beef is gotten from animals between the ages of three to four years. The use of antimicrobial agents in livestock is fast growing [2] and several reasons have been put forward to justify this including to avert and treat infections or to enhance growth [3].

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Many of these antimicrobial agents are similar to the antimicrobials used in humans. In the production of livestock, separate animals may be treated; however it is usually more cost effective to treat whole groups by introducing drugs into their feed or water. For certain animals, like fish and poultry, bulk medication is the most practicable way of treatment [4]. World-wide ingestion of antimicrobials in livestock production had been estimated at 63,151 (±1,560) tons in 2010 and is anticipated to increase by 67%, to 105,596 (±3,605) tons, by 2030 [2].

The risk to human wellbeing following wrong antibiotic use in food animals is noteworthy and has become a subject of public health discuss among scientists, especially in the developing countries. Pathogenic-resistant organisms

propagated in these livestock are poised to enter the food supply and could be widely disseminated in food products posing serious danger to public health.

Antimicrobial resistance has been partly attributed to the extensive use of antimicrobials in livestock. It is opined by some researchers that, such resistance largely occur through natural selection with its attendant consequences to public health [2]. The occurrence of drug resistance has been detected after the introduction of every new class of antimicrobial agents. This danger is compounded by a dawdling drug development channel and insufficient investment in the innovation and development of novel antimicrobial agents. Commensal bacteria found in livestock are frequently present in fresh meat products and may serve as reservoirs for resistant genes that could potentially be transferred to pathogenic organisms in humans [5].

Although previous studies have reported contamination of beef with *Salmonella typhi* and *Listeria monocytogenes*, in other parts of Nigeria and worldwide, there is no data on the presence of these pathogenic bacteria in raw beef sold in Abuja, North Central Nigeria. *Salmonella* is a foremost food borne pathogen, responsible for an estimated 98.3 million cases of gastroenteritis worldwide [6]. This organism has frequently been associated with poultry [7]. However, other sources of meat such as pork and lamb have also been implicated as sources of contamination [8, 9]. The pathogen *Listeria* has been recognized as the causative agent of Listeriosis. Annually, this organism is estimated to cause 2,500 illnesses and 500 associated deaths [10]. Typical foodstuff implicated as sources of the organism include salad, cooked and fermented meat and raw meat such as beef, pork, lamb, poultry [11, 12, 9]. Usually a *Listeria* infection is linked to a well-defined high risk groups such as the immuno-compromised or pregnant women [13].

Timely recognition of emerging infections requires early warning systems to detect problems so that they may be promptly investigated and controlled before they evolve into public health crises. Adequate data in this area of research is not readily available in Nigeria; this study was undertaken in view of the possible link between antimicrobial resistance in farm animals and humans. This study was designed to investigate the presence of *Salmonella typhimurium* and *Listeria monocytogenes* on beef, its antibiotics susceptibility and thermal resistance during subsequent heat treatment.

MATERIALS AND METHODS:

Antibiotics: All antibiotic discs were obtained from Oxoid, UK. Amoxicillin / clavulanic acid (20/10 µg), nalidixic acid (30 µg), tetracycline (30 µg), gentamycin (10 µg), streptomycin (10 µg), choramphenicol (30 µg), erythromycin (15 µg), cefuroxime (30 µg), nitrofurantoin (300 µg), and ciprofloxacin (5 µg).

Media:

All media were obtained from Oxoid, UK. Xylose Lysine Deoxycholate agar (XLD), Tetrathionate broth, Oxford agar, Listeria selective broth, Nutrient agar, buffered peptone water, Sabouraud dextrose agar, API test kits.

Sampling procedure:

Fifty raw beef samples were procured at random from vendors in slaughter houses and meat stalls located in the Six Administrative Councils of the Federal Capital Territory, Abuja, Nigeria. The samples were transported to the laboratory in sterile bags. Analysis was carried out within 6 hours after sampling. However, where immediate microbiological evaluation was to be delayed, the samples were refrigerated at 4°C and analyzed within 24 hours of collection [14].

Processing of samples:

Twenty-five grams each of the meat sample was weighed aseptically and immersed in 225 mL buffered peptone water. These meat samples were kept in the incubator 37 °C for 24 h. One milliliter of ten-fold serially diluted sample was inoculated on nutrient agar for bacteria enumeration and incubated at 37 °C for 24 h and onto Sabouraud dextrose agar for fungal enumeration at 25 °C for 72 h. All bacterial and fungal counts were recorded as cfu/g.

Isolation and identification of *Salmonella typhimurium*: The method by Erol *et al*. (2013) [15]

was adopted with slight modifications; simply, 1 mL of pre-enriched culture was transferred into 9 mL of tetrathionate broth supplemented with freshly prepared iodine solution and incubator at 37°C for 24 h. Presumptive *Salmonella* spp colonies were streaked onto xylose lysine deoxycholate (XLD) agar and incubated at 37 ± 2 °C for 18-24 h.

Typical black colonies with yellow halo were picked and archived on nutrient agar slants until when needed. For identification, presumptive *Salmonella typhimurium* were streaked onto tryptone soy agar supplemented with 5 % sheep's blood on soy agar and incubated at 37 °C for 24 h. Confirmatory tests included: Gram stain, triple sugar agar tests, urease tests and sugar tests via API 20E test kits. Isolates showing reactions typical of *Salmonella* were confirmed by agglutination tests.

Isolation and identification of *Listeria monocytogenes*:

The method by Moustafa *et al*., (2011) [16] was adopted with slight modifications; one millilitre of the pre-enriched culture was added to 9 mL of *listeria* selective broth and further incubated for 24 h at 30 °C. The broth culture (after incubation) was streaked onto plates of Oxford agar and incubated at 37 °C for 48 h. Gray colonies surrounded with black centres were presumed to be *Listeria monocytogenes*. Presumptive *Listeria* colonies were picked and purified by streaking onto tryptone soy agar supplemented 5 % sheep's blood. Non sporolating Gram positive *cocobacilli* were subjected to further identification tests: motility test, CAMP test, haemolysin production, fermentation of xylose, rhamnose, mannitol.

Susceptibility Testing:

Susceptibility test was performed according to Bauer-Kirby method of disc diffusion using Muller Hinton agar [17]. Overnight culture of pure isolates grown on appropriate solid media was inoculated into sterile normal saline with turbidity matching 0.5 McFarland standard. The standardized culture was then streaked on the surface of Muller Hinton agar and the inoculated plates allowed to stand for 30 min, before standard antibiotic discs were placed equidistantly from each other using sterile forceps. The plates were inverted and incubated at 37^{o}C for 24 h. Isolates resistant to at least three different classes of antibiotics were termed multi-drug resistant (MDR) [18].

Thermal resistance testing:

Glass tubes containing the heating menstruum (9 mL tryptic soy broth) were placed in different water baths at 55, 60, 65 and 70 °C. When the heating menstruum reached the desired temperatures, one millilitre of standardized culture of multi drug resistant isolates were inoculated into the glass tubes containing the heating menstruum such that the initial counts were 1.0 x 10^{7} -1.0 x 10^{8}cfu/mL.

The menstruum in the tubes was heated for 5, 10 and 15 minutes. Enumeration of organism surviving the heating was carried out on the appropriate media, after incubation at 37 °C for 48 h. The number of organisms surviving was plotted against the heating times to yield a curve of rate of inactivation at four different temperatures. Based on the curve, the D values, i.e. time (minutes) at certain temperatures to reduce the number of organism by 1 log cycle was calculated from the equation D = -1/slope.

A Thermal Death Time (TDT) curve was made to establish the relationship between D (minutes) with temperatures (^{o}C). The Z values, i.e. temperature intervals to reduce D value by 1 log cycle was also determined from the curve [19].

RESULTS:

Confirmation of microorganisms:

The results of the biochemical and confirmatory tests confirmed 10 isolates to be pure cultures of *Listeria monocytogenes* while 18 isolates were confirmed to be pure cultures of *Salmonella typhimurium* (**Table 1**). Isolates were designated as Q for *Salmonella* isolates and P for *Listeria*. Initial count for *Salmonella* ranged from 1.5 – 2.4 x 10^{10} with Q3 and Q9 showing the highest number of counts with 2.4 × 10^{10}cfu/g while initial count for *Listeria monocytogenes* ranged from 1.4 – 2.2 x 10^{10}cfu/g with P5 showing the highest number of counts of 2.2 × 10^{10}cfu/g (**Table 2**).

TABLE 1: FREQUENCY AND PREVALENCE RATE OF THE ISOLATED PATHOGENS

Bacteria Isolated	Number of Isolates	Prevalence (%)
Listeria monocytogenes	10	36
Salmonella typhimurium	18	64
Total	**28**	

TABLE 2: INITIAL TOTAL COUNTS OF *SALMONELLA TYPHI AND LISTERIA MONOCYTOGENES* IN BEEF SAMPLES COLLECTED.

***Salmonella* Typhi**		***Listeria monocytogenes***	
Isolates	**Total viable count(cfu/g)**	**Isolates**	**Total Viable count(cfu/g)**
Q1	1.8×10^{10}	P1	1.4×10^{10}
Q2	2.0×10^{10}	P2	1.9×10^{10}
Q3	2.4×10^{10}	P3	2.0×10^{10}
Q4	1.9×10^{10}	P4	1.9×10^{10}
Q5	2.2×10^{10}	P5	2.2×10^{10}
Q6	2.0×10^{10}	P6	1.8×10^{10}
Q7	1.8×10^{10}	P7	1.5×10^{10}
Q8	1.5×10^{10}	P8	1.6×10^{10}
Q9	2.4×10^{10}	P9	1.6×10^{10}
Q10	2.2×10^{10}	P10	1.5×10^{10}
Q11	2.0×10^{10}		
Q12	1.9×10^{10}		
Q13	2.1×10^{10}		
Q14	1.8×10^{10}		
Q15	2.0×10^{10}		
Q16	2.3×10^{10}		
Q17	1.9×10^{10}		
Q18	1.8×10^{10}		

Antibiotics susceptibility of isolated bacteria: Drug susceptibility assay revealed that isolate P_1 was resistant to all antibiotics tested. Cefuroxime was the least effective antibiotic tested, as it inhibited only the growth of isolate P4. Gentamicin inhibited the growth of isolates P1 and P3 (8/10) while chloramphenicol inhibited all except P4 and P10 (2/10). Isolate P4 was susceptible to all the antibiotics tested, while the other isolates were resistant to one or more of the antibiotics used **(Table 3**). Drug susceptibility assay showed that streptomycin and gentamicin were the most effective in inhibiting the growth of all *Salmonella* isolates, followed by ciprofloxacin, nalidixic acid nitrofurantoin, tetracycline and chloramphenicol, while cefuroxime showed the least efficacy against the isolates. Isolate Q14 showed the highest resistance to the antibiotics, while Isolates Q4 and Q5 were most susceptible to all antibiotics tested (**Table 4).**

TABLE 3: ANTIBIOTICS SUSCEPTIBILITY TEST FOR ISOLATED LISTERIA MONOCYTOGENES

Isolates	**Diameter of zones of inhibition to different antibiotics (mm)**						
	Gen(10µg)	Cxm(30µg)	Ery(15µg)	Tet(30µg)	Cip(5µg)	Cam(30µg)	Amx(20µg)
P1	-	-	-	-	-	-	-
P2	15	-	-	-	12	-	-
P3	-	-	24	16	22	-	-
P4	37	26	36	22	34	24	27
P5	20	-	11	-	17	-	-
P6	15	-	22	-	-	-	-
P7	23	-	-	10	10	-	-
P8	24	-	13	15	-	-	-
P9	29	-	-	15	20	-	18
P10	18	-	22	-	19	11	21

Keys: Gen = gentamicin, Cxm = cefuroxime, Ery = erythromycin, Tet = tetracycline, Cip = ciprofloxacin, Cam = chloramphenicol, Amx = amoxicillin, - = no inhibition.

TABLE 4: ANTIBIOTICS SUSCEPTIBILITY TEST FOR ISOLATED *SALMONELLA*.

Isolate	Diameter of zones of inhibition to different antibiotics (mm)							
	Str (10µg)	Nal (30µg)	Cip (5µg)	Cxm (30µg)	Tet (30µg)	Cam (30µg)	Gen (10µg)	Nit (300 µg)
Q1	22	-	22	-	15	19	22	-
Q2	17	16	31	-	19	18	37	11
Q3	15	10	33	-	-	-	31`	11
Q4	16	22	32	15	24	27	28	23
Q5	14	10	26	10	21	11	28	10
Q6	20	17	30	19	18	-	28	15
Q7	15	10	32	-	19	15	31	18
Q8	19	17	29	-	20	10	29	17
Q9	23	17	26	-	19	-	31	21
Q10	24	19	31	19	20	-	25	19
Q11	15	11	29	-	19	-	27	10
Q12	18	11	26	-	-	17	25	10
Q13	14	17	29	-	19	-	29	15
Q14	10	13	-	-	15	-	25	-
Q15	15	17	26	-	15	-	27	23
Q16	10	19	33	10	-	12	32	10
Q17	19	15	31	-	-	-	26	17
Q18	17	19	28	-	15	11	27	27

Keys: Str = streptomycin, Nal = nalidixic acid, Cip = ciprofloxacin, Tet = tetracycline, Cam= chloramphenicol, Gen = gentamicin, Nit = nitrofurantoin, - = no inhibition.

Heat treatment of *Listeria monocytogenes* and *Salmonella typhi* isolates: Tables 5 and **6** show the colony count of surviving cells of *Listeria monocytogenes* and *Salmonella typhi* after being subjected to varying degrees of heat at 5, 10 and 5 minutes respectively. Generally, cell death increased with increase in temperature. All isolates of *listeria* except P7 were killed upon exposure to heat at 60 ^{o}C for 5 minutes. For *Salmonella*, isolates Q3 and Q10 were the most resistant to heat, and were observed to survive at 60 ^{o}C for 15 minutes.

TABLE 5: EFFECT OF HEAT TREATMENT ON *LISTERIA MONOCYTOGENES* ISOLATE

Isolate	Number of viable bacteria after exposure to heat								
		50^{o}C			55 ^{o}C			60 ^{o}C	
	RT	5′	10′	15′	5′	10′	15′	5′	10′
P1	145	90	20	5	-	-	-	-	-
P2	190	100	50	20	2	-	-	-	-
P3	200	140	70	35	10	-	-	-	-
P4	198	95	30	10	3	-	-	-	-
P5	220	150	90	30	5	-	-	-	-
P6	180	110	60	15	1	-	-	-	-
P7	150	85	50	15	7	1	-	-	-
P8	160	92	30	10	2	-	-	-	-
P9	162	92	30	10	2	-	-	-	-

TABLE 6: EFFECT OF HEAT TREATMENT ON *SALMONELLA TYPHI* ISOLATES

Isolate	Number of viable bacteria after exposure to heat								
		50^{o}C			55^{o}C			60^{o}C	
	RT	5′	10′	15′	5′	10′	15′	5′	10′
Q1	180	120	80	35	5	1	-	-	-
Q2	200	160	102	50	15	2	-	-	-
Q3	235	195	105	60	25	4	1	-	-
Q4	192	105	72	20	8	3	-	-	-
Q5	220	180	100	40	5	-	-	-	-
Q6	200	155	100	45	10	-	-	-	-

Q7	182	100	70	42	15	5	-	-	-
Q8	150	90	30	5	1	-	-	-	-
Q9	240	190	110	70	21	7	-	-	-
Q10	220	172	105	65	18	9	2	-	-
Q11	200	150	100	50	10	-	-	-	-
Q12	190	100	60	35	12	4	-	-	-
Q13	210	170	105	55	15	7	-	-	-
Q14	185	105	72	38	5	1	-	-	-
Q15	200	160	100	50	10	-	-	-	-
Q16	230	180	92	40	2	-	-	-	-
Q17	195	100	60	25	4	-	-	-	-
Q18	180	130	90	42	10	-	-	-	-

Decimal reduction time (D-value) of isolates: The D values of *Listeria monocytogenes* and *Salmonella typhi* for five minutes at 55 and 60 ^{o}C are shown in tables 7 and 8. There was a decrease in the values as temperature increased**.** Isolate P8 of *Listeria* was the only colony that retained its viability at 55 ^{o}C with a D value of 32.67 S, while *Salmonella typhi* isolates Q3 and Q10 were viable at 55 ^{o}C with D values of 32.01 and 33.18 seconds respectively.

TABLE 7: DECIMAL REDUCTION VALUE FOR *LISTERIA MONOCYTOGENES*

		Decimal reduction time							
		50 ^{o}C			55 ^{o}C			60 ^{o}C	
Isolate		5′	10′	15′	5′	10′	15′	5′	10′
P1	145	41.61	38.16	35.46	-	-	-	-	-
P2	190	41.21	39.57	37.59	33.41	-	-	-	-
P3	200	41.96	40.26	38.65	36.14	-	-	-	-
P4	198	40.98	38.36	36.14	34.01	-	-	-	-
P5	220	41.90	40.59	38.16	34.72	-	-	-	-
P6	180	41.55	40.10	37.12	32.40	-	-	-	-
P7	150	41.38	40.10	37.50	36.01	32.67	-	-	-
P8	160	41.67	39.73	37.40	35.29	-	-	-	-
P9	162	41.44	38.86	36.58	33.71	-	-	-	-

TABLE 8: DECIMAL REDUCTION VALUE FOR *SALMONELLA TYPHI*

		Decimal reduction time							
		50 ^{o}C			55 ^{o}C			60 ^{o}C	
Isolate		5′	10′	15′	5′	10′	15′	5′	10′
Q1	180	41.78	40.76	38.86	35.05	32.39	-	-	-
Q2	200	42.25	41.15	39.47	36.95	33.33	-	-	-
Q3	235	42.37	40.81	39.52	37.64	34.20	32.01	-	-
Q4	192	41.32	40.43	37.59	35.80	34.09	-	-	-
Q5	220	42.37	40.87	38.75	34.72	-	-	-	-
Q6	200	42.19	41.09	39.21	36.14	-	-	-	-
Q7	182	41.32	40.48	39.26	37.13	35.04	-	-	-
Q8	150	41.49	38.96	35.37	32.68	-	-	-	-
Q9	240	42.25	40.87	39.84	37.22	35.16	-	-	-
Q10	220	42.25	40.98	39.84	37.13	35.75	33.18	-	-
Q11	200	42.13	41.09	39.42	36.14	-	-	-	-
Q12	190	41.21	40.00	38.75	36.59	34.56	-	-	-
Q13	210	42.31	41.09	39.57	36.86	35.41	-	-	-
Q14	185	41.38	40.48	39.01	35.01	32.36	-	-	-
Q15	200	42.25	41.09	39.47	36.14	-	-	-	-
Q16	230	42.25	40.54	38.65	33.11	-	-	-	-
Q17	195	41.15	39.94	38.02	34.52	-	-	-	-
Q18	180	41.96	41.03	36.31	36.32	-	-	-	-

DISCUSSION: Food borne disease caused by *Listeria monocytogenes* and *Salmonella typhi* represent a major public health problem especially in the developing countries where infectious diseases predominates due largely to poor or inadequate health facilities and hygiene. It has been established that these pathogens are transmitted through contaminated livestock products such as beef. Contamination of beef begins during slaughter of the animal due to poor hygienic conditions and handling processes of the slaughter houses or abattoir. The reliance on beef as a source of dietary proteins enhances the transfer of these disease causing pathogens to humans [20]. The consumption of contaminated meat and meat products is therefore a major vehicle in the transfer of pathogenic organisms to man.

In this study, it was evident from the bacteriological analysis of the investigated samples that *Salmonella typhimurium* and *Listeria monocytogenes* contaminate a large percentage of meat products. We discovered that 36 % (18/50) of all samples collected were positive for *Salmonella* and 20 % (10/50) were positive for *Listeria monocytogenes*. Compared to other studies, we report higher levels of *Salmonella* contaminants in meat samples. Lukasz *et al.* (2014) [21], showed that 10.4 % (11/106) of meat products were found to be positive for *Salmonella*. Ukut *et al*., (2010) [22] reported that 11 % meat samples from Calabar were contaminated with *Salmonella*, Adesiji *et al*., (2011) [23] reported 2 % contamination in samples from Oshogbo.

Tafida *et al*., (2012) [24] showed that *Salmonella* was isolated from 2.93 % of meat samples collected from Zaria. We also showed that Listeria was present in 35 % of interrogated samples. *Listeria monocytogenes* has also been found in different kinds of raw meat including beef, 20.8 % [25] and 30 % of samples [26]. Previously, Daniel *et al*., (2015) [27] reported a 17.15 % rate contamination for Listeria in fresh and frozen chicken from markets in Makurdi, Nigeria. This larger contamination levels recorded for Abuja may be attributed to its strategic national status in Nigeria; being the administrative headquarter of Nigeria, the city plays host to the 3 arms of government, foreign diplomats as well as national and international investors. This cosmopolitan nature of the city encourages increase importation and consumption of varieties of meat and meat products in Abuja. According to the Centre for Disease and Control (CDC) [28,] with increasing resistance to fluoroquinolones and third-generation cephalosporin, *Salmonella* is now responsible for about 94 million cases of gastroenteritis and 115,000 deaths globally. It has been postulated that although *Listeria* is not as common as other food borne pathogens such as *Salmonella* and *Escherichia coli*, it is one of the deadliest and adaptable bacteria found in food[29]. It is reported to cause up to 23,150 infections and 5,463 deaths worldwide.

The antibiotics susceptibility result for our study indicated resistance frequencies for *Salmonella typhimurium* and *Listeria monocytogenes.* We observed that most *Salmonella* isolates were resistant to one or more of the antibiotics, while isolates Q4 and Q5 showed no resistance to the antibiotics tested. Streptomycin and gentamicin inhibited the growth of all 18 *Salmonella* isolates. Previous reports by Kakatka *et al*., (2011) [30] have also found *Salmonella typhimurium* isolates from Indian foods to be sensitive to ampicillin and ciprofloxacin. Our data showed that all isolates of *Listeria* except P4 showed resistance to one or more of the tested antimicrobial drugs, this observation is consistent with previous studies such as those of Rahimi *et al* (2012) [31]; Carmago *et al* (2015) [32] and Wieczorek *et al* (2012) [33]. These authors reported significant levels of antimicrobial resistant *Listeria* isolates from meat and hides circulating in Iran, Brazil and Poland respectively. Cefuroxime showed the lowest efficacy against *Salmonella* and *Listeria* isolates.

The low efficacy of cefuroxime against the isolates may be attributed to the easy hydrolysis of the β-lactam ring by most bacteria [34]. On the other hand, gentamycin was most effective in inhibiting the growth of the isolates. Gentamycin, a bactericidal aminoglycoside acts by irreversibly binding to the 30S subunit of bacterial ribosome, interrupting protein synthesis in the bacteria leading to cell death[35].

Food borne pathogens can acquire resistance in response to antimicrobial drug use in food and

animal contaminated food products at the time of slaughter and possibly transmit the resistant genes to human via the food chain [36]. According to Narfarnda *et al*., (2012) [37] administration of antimicrobial drugs close to time of slaughter leads to the occurrence of antimicrobial residues in up to 89 % of animal tissue in beef samples from Abuja. The presence of pathogens resistant to a number of antibiotics as reported in this study can be linked to residual antibiotics in the animal tissues. The persistence of antimicrobial drug residues is a possible factor in driving antimicrobial resistance in bacteria isolated from livestock. A call by the US FDA for prudent use of antibiotics in both human and animal medicine has been issued for years with some positive results (FDA, 2000) [38]. In 2005 US Food and Drug administration (FDA) placed a ban on the use of enrofloxacin (a fluoroquinolone drug structurally related to ciprofloxacin) in poultry because of the risk that it promotes drug-resistant bacteria that are harmful to human health.

We discovered and report herein a high incidence of *Salmonella* and *Listeria* in beef being consumed in Abuja, the Federal Capital City of Nigeria. It is recommended that meat products purchased from markets should be cooked thoroughly to kill bacteria and toxins before consumption as earlier suggested by Adeyanju and Ishola (2014) [39]. In order to establish scientific evidence and justify the suggestion to cook the products well before consumption, the isolates from our study were subjected to heat treatment to analyse the effect of heat on the viability of isolated bacteria. None of the isolates of *Salmonella* or *Listeria* survived beyond exposure to heat at 55^{o}C.

An average figure for the D values of *Salmonella* at D55 and D60 were 41.90 and 35.84 seconds respectively, while *Listeria* had corresponding average values of 41.52 and 30.63. Our observation is consistent with that of Murphy, *et al.,* (2004) [40]. In their study, the D value for *Salmonella* at 55 and 70^{o}C were reported to be 43.33 and 43.76 s respectively, while the corresponding values for *Listeria monocytogenes* were 38.94 and 34.05 s. In a separate study on ready-to-eat turkey bologna by McCormwick *et al*., (2012) [41], that the D values for *Salmonella typhimurium* isolates at 57 and 60 ^{o}C were reportedly 278 and 57 s respectively, while D values for *L. monocytogenes* at 61 and 65 ^{o}C were 124 and 16.2 s respectively. The differences between D values in this study and those previously reported may be due to different bacteria species, physiological conditions of the cells or use of cultures at different growth phases as previously argued by Juneja and Eblen (2002) [42].

CONCLUSION: We confirm the role of raw beef as a reservoir of not just microorganism but also of microorganisms that are antibiotics resistant. The results of this study show intermediate to high resistance to antimicrobial drugs in isolates of *S. typhimurium* and *L. monocytogenes* from beef marketed in Abuja. We suggest that this high resistance to antimicrobial drugs also correlated with increased survival when exposed to heat. The application of hygiene practices in meat processing centres and the prudent use of antibiotics in animal husbandry are therefore essential to control further emergence and transmission of antibiotic resistance.

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REFERENCES:

1. Oluwafemi R A, Edugbo O M, Solanke E O and Akinyeye A J:Meat quality, nutrition security and public health: a review of beef processing practices in Nigeria.African Journal of Food Science and Technology 2013; 4(5):96-99.
2. Van Boekkel TP: Global Trends in Antimicrobial Use in Food Animals. Proc. Natl. Acad. Sci. USA 2015; 112: 5649-5654.
3. Mcewen CA and Fedorka-Cray PJ: Antimicrobial Use and Resistance in Animals. Clin. Infect. Disease 2002; 34(3): 1-5.
4. American Veterinary Medical Association. Judicious therapeutic use of antimicrobials. Available at: http://avma.org/scienact/jtua/ jtua98.asp. Accessed 13 January 2016.
5. Mena C, Almeida G, Carneiro L, Teixeira P, Hogg T, Gibbs PA: Incidence of L. monocytogenes in Different Food Products Commercialized in Portugal; Food Microbial 2004; 21: 231-216.
6. Poudel S, Shresthas K, Prandhan A, Sapkota B, Mahota : Antimicrobial Susceptibility Pattern of S enterisspp in Blood Culture Isolates. Clin. Microbial 2014; 3(14): 1-4.
7. Bryan, F. and Doyle, M. P: Health risk and consequences of *Salmonella* and *Campylobacter jejuni* in raw Poultry. Journal of food Protection 1995; 3:299-344.

8. Duffy, G. Cloak, O. M., O'Sullivan, M. G., Guillet, A., Sheridan, J. J., Blair, I. S., McDowell, D. A: The incidence and antibiotic resistance of Salmonella spp. on Irish retail meat product. Food Microbiology 1999; 16: 623-631.
9. Madden, R. H., Epie, W. E., Moran, L., McBride, J. and Scates, P: Occurrence of *E. coli*.0157:H7, *Listeria monocytogenes*, *Salmonella* spp. and *Camplylobacter* spp on beef carcasses in Northern Ireland. Meat Sciences. 2001; 58: 343-346.
10. Mead, P. S., Slusker, L., Dietz, V., Mccaig, L. F., Bresee, J. S., Shapiro, C. Griffin, P. M. and Tauxe, R. V: Food related illness in the United States. Emerging Infectious Diseases. 1999; 5: 607-625.
11. Hudson, J. A., Mott, S. J. Delacy, K. M. and Edridg, A. L: Incidence and coincidence of Listeria spp. Mortileaeromonads and Yersinia enterocolitica on ready to eat flesh food. International Journal of Food Microbiology 1992; 2:99-108.
12. Sheriddan, J. J., Duffy, G., McDowell, D. A. and Bliar, I. S: The occurrence and initial numbers of Listeria in Irish meat and fish Products and the recovery of injured cells from frozen products. International Journal of Food Microbiology 1994; 22:105-113.
13. Swaminathan, B. Listeria monocytogenes in food microbiology. Fundamentals and frontiers. 2nd edtion, American Society of Microbiology Press. Washington D. C. 2001; 383-409.
14. Salihu MD, Magaji A, Garba B, Saidu B, Mamudu A, Suleiman N, Warno BS: Bacteriological Quality of Raw Meat Displayed for Sale at Sokoto, Sokoto State, Nigeria. Sci J. Microbiol. 2013; 2(7): 1-5
15. Erol I, Lioncuoghi M, Ayaz ND, Ellerbroek L, Ormanic FSB, Kangal OI: Serotype distribution of *Salmonella* isolates from turkey ground meat and meat parts; Biomed. Res. Int. 2013; 1-5.
16. Moustafa El Shenaway, Mohamed El-Shenaway, Jose M Soriano, Jodri Manes: *Listeria*spp in street-vended ready-to-eat Foods. Interdisciplinary Perspectives on Infectious Disease; 2011; 1-6.
17. Bauer AN, Kirby WM, Sherries JC, Turck M: Antibiotic Susceptibility Testing by a Standardized Single Disk Method. Amer. J. Clin. Pathol 1966; 45:493-496.
18. Chen, S., Zhao, S., White D.G., Schroeder, C.M., Lu, R., Yang, H., et al: Characterization of multiple-antimicrobial-resistant Salmonella serovars isolated from retail meats. Appl. Environ. Microbiol. 2004; 70(1): 1-7.
19. Ratih, D.W, Juli, H. and Eko, H. P. (). Thermal resistance of local isolates of Staphylococcus aureus. Asian. Journal of Food and Agro Industry 2011; 4(04):213-221.
20. Agarry, O. O., Ugoh, S. C., and Abeku M: Antimicrobial potentials of some spices on beef sold in Gwagwalada marker, FCT, Abuja. *Report and Opinion* 2011; 3(1): 96-98.
21. Ukut, I. O., Okonko, I. O., Ikpoh, I, S., Nkang, A. O., Udeze, A. O., Babalola, T. A., Mejeha, O. K. and Fajobi, E. A: Assessment of bacteriological quality of fresh meats sold in Calabar metropolis, Nigeria. *Electronic Journal of Environmental Agricultural and Food Chemistry* 2010; 9(1):89–100.
22. Adesiji Y. O., Alli, O.T., Adekanle, M.A., Jolayemi, J.B: Prevalence of Arcobacter Escherichia coli, Staphylococcus aureus and Salmonella species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. *Sierra Leone Journal of Biomedical Research* 2011; 3(1):8-12.
23. Tafidat, S.Y., Kabir, J., Kwanga, J. K. P., Bello, M., Umoh V. J., Yakubu, S. E., Nok, A. J., Hendriksen R: Occurrence of Salmonella in retail beef and related meat products in Zaria, Nigeria. *Food control*. 2012; 33(1): 119-124.
24. Sramora H, Benes C, Karpiskova R: Listerioses in the Czech Republic and around the World (In Czech). The Bulletin of Centre of Epidemiology and Microbiology Prague; 2000; 9:363-365.
25. Amoral JG and Bhunia AK. Immunological and Cytopathogenic Properties of L. monocytogenes Isolated from Naturally Contaminated Meats. Journal of Food Safety 1999; 19(3): 195-207.
26. Daniel S. T., Umeh E. U. and Iheukwumere C. C: Contamination and Antibiotic Susceptibility Profile of Listeria Species in Frozen and Fresh Chicken Sold in Makurdi, Nigeria. *International Journal of Current Microbiology and Applied Sciences*. 2015; 4(7): 617-623.
27. Centre for Diseases Control and Prevention: National Antimicrobial Resistance Monitoring System (NARMS) for enteric bacteria human isolates final report. Atlanta, George. U.S Department of Health and Human Services. 2006
28. Maertens De Noordhout C, Devleesschauwer B, Angulo FJ, Verbeke J, Haagsma J, Kirk M, Havelaar A, Speybroeck N: The global burden of listeriosis: a systematic review and meta-analysis., The Lancet Infectious Diseases 2010; 14(11): 1073-1082.
29. Vázquez-Boland JA, Kuhn M, Berche P, Chakraborty T, Domínguez-Bernal G, Goebel W, González-Zorn B, Wehland J and Kreft J. *Listeria* Pathogenesis and Molecular Virulence Determinants. ClinMicrobiol Rev. 2001; 14(3): 584–640. doi: 10.1128/CMR.14.3.584-640.
30. Kakattar A, Pansare LS, Gautam RK, Shashidhar R, Karani M, Randekar T. Molecular Characteristics of Antibiotic Resistance in Salmonella Isolated from Indian Foods; Food Res Inter 2011; 44(10): 3272-3275.
31. Rahimi, E., Yazdi, F and Farzinezhadizadeh, H: Prevalence and antimicrobial resistance of listeria species isolated from different types of raw meat in Iran. Journal of Food Protection 2015; 75(12):2223-2227.
32. Camargo, A. C., de Castilho, N. P., da Silva, D. A., Vallim, D. C., Hofer, E. and Nero, L. A: Antibiotic Resistance of Listeria monocytogenes Isolated from Meat-Processing Environments, Beef Products, and Clinical Cases in Brazil. Microbial Drug Resistance 2015; 21(4): 458-462.
33. Wieczoreck, K., Dmowska, K. and Osek, J: Prevalence, characterisation, and antimicrobial resistance of Listeria monocytogenes isolates from bovine hides and carcasses. Applied Environmental Microbiology 2012; 78(6): 2043-2045.
34. Adeshina, G. O., Jibo, S. D., Agu, V. E: Antibacterial Susceptibility Pattern of Pathogenic Bacteria Isolates from Vegetable Salad Sold in Restaurants in Zaria, Nigeria. Journal of Microbiology Research 2012; 2(2):5-11.
35. Kadurugamuwa JL, Clarke AJ and Beveridge TJ: Surface action of gentamycin on *Pseudomonas aeruginosa*. J. Bacteriol. 1993; 175(18): 5798-5805.
36. Bower, C.K. and Daeschel, M.A: Resistance responses of microorganisms in food environments. Intl. J. Food Microbiol. 1999; 50:33-44.
37. Narfarnda WD, Ajayi IE, Shawalu JC, Kawe MS, Omeiza GK, Sani NA, Tenuche OZ, Dantong DD, Tags SZ. Bacteriological quality of abattoir effluents discharged into water bodies in Abuja. Veterinary Science 2012; 515689: 1-5. doi:10.5402/2012/515689.
38. Food and Drug Administration Center for Veterinary Medicine: An approach for establishing thresholds in association with the use of antimicrobial drugs in food-

producing animals. Rockville, MD: Food and Drug Administration Center for Veterinary Medicine. 2000.
39. Adeyanju, G., T. and Ishola, O: *Salmonella* and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. SpringerPlus 2014; 139 (3):1-9.
40. Murphy RY, Osaili T, Duncan LK, Mercy JK: Salmonella and *Listeria monocytogenes* in Ground Chicken Thigh/Leg Meat and Skin. Poultry Science 2004; 83(7): 1218-1225
41. McCormick, K., Han, I. Y., Acton, J. C., Sheldon, B. W., Dawson, P. L: D and z-values for *Listeria monocytogenes* and *Salmonella typhimurium* in packaged low-fat ready-to-eat turkey bologna subjected to a surface pasteurization treatment. Poultry Science 2003; 82(8): 1337-42.
42. Juneja, V. K., Eblen, B.S. Heat inactivation of *Salmonella typhimurium* DT104 in beef as affected by fat content. Letters in Applied Microbiology 2002; 30(6):461-461.

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