BACTERIOLOGICAL QUALITY OF BEEF AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF ISOLATES FROM TWO REGIONAL SLAUGHTERHOUSES IN NIGERIA

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Abstract
Slaughtering methods and practices in Sub-Saharan African countries tend to contribute to the bacterial quality of meat and could be a source of bacterial contamination of meat due to noncompliance with the National Environmental Health Practice Regulations. This study aimed to assess the microbial quality of beef and antimicrobial susceptibility profiles ofisolates from Nsukka and Owerri abattoirs. A completely randomized and a cross-sectional design were adopted. A total of 240 samples from two slaughterhouses (120 meat samples from each slaughterhouse) were collected during 15 visits. The mean aerobic plate and coliform counts of Nsukka meat and Owerri meat abattoirs from the table were significantly ($p<0.05$) higher than those of Nsukka and Owerri abattoirs from the floor, respectively. Verotoxigenic E. coli and Salmonella spp were isolated from the meat on the floor of both abattoirs. The E. coli and Salmonella isolated from both slaughterhouses (Nsukka and Owerri) were resistant to neomycin (57.15%, 40.00% and 53.06%, 14.29%), gentamicin (14.29%, 20.00% and 6.12%, 0%), amoxycillin (100%, 100% and 100%, 66.6%), tetracycline (42.86%, 40.00% and 83.67%, 66.60%), doxycycline (14.29%, 0% and 61.22%, 0%), nitrofuratoin (42.86%, 40.00% and 22.45%, 42.86%), streptomycin (100%, 100% and 83.67%, 71.43%), amoxicillin-clauvulamic acid (100%, 0% and 93.88%, 7%), ciprofloxacin (28.58%, 20.00% and 38.32%, 28.58%) sulphamethazole/trimethoprin (28.57%, 20% and 46.94%, 14.29%). High microbial loads and pathogenic microorganisms have been found in both abattoirs, indicating unsanitary practices. On the other hand, the slaughtering and processing of animals should be improved, regulated, and enforced by the appropriate authorities.

Keywords: abattoir, aerobic bacteria, antibiogram, meat, plate count

Received: 10.02.2022 Reviewed: 29.03.2022 Accepted: 30.03.2022

1. INTRODUCTION

Meat such as beef is a major component of the human diet and has a high-water activity (0.99) and adequate nutrient for microbial growth (Rao *et al*., 2009; Chuku *et al*., 2016). Microbe's growth in meat can cause visual, textural and organoleptic changes as the microbes release metabolites that reduce the meat's quality (Rao, *et al*., 2009; Okechukwu *et al*., 2018). Possible sources of the bacteria include, in particular, the animal's skin from which the meat is obtained, clothing, personnel and the equipment used for each operation in slaughterhouse (Chuku *et al*., 2016). Unfortunately, in most developing countries, the slaughterhouses are below standard level and this has a significant impact on the microbial quality of meat (Bello and Son, 2009; Gali *et al.* 2020). The bacterial genera most commonly contaminated meat during slaughter are *Salmonella, Campylobacter, Escherichia*, *Staphylococcus, Clostridium, Klebsiella, Proteus, Bacillius,* and *Listeria.Salmonella* and Furthermore *Escherichia coli*aremost commonly found in animal body coat and faeces (Finch and Hunter 2006; Tenover, 2006). Antimicrobial drugs are commonly utilized in veterinary medicine for the disease prevention and treatment (Anderson *et al*., 2003). The use of antimicrobials in animal production could cause overtime precipitation of multidrug resistant strains of *Salmonellae* and *Escherichia coli* (Finch and Hunter 2006).

Salmonella and Verotoxygenic *E. coli* strains are especially of great public health concern, for the treatment of these infections is more difficult when resistance is encountered (Tenover, 2006). In Sub-Sahara African country, the abattoir environment, slaughtering methods and practices tend to contribute to the bacterial quality of meat and slaughter operations are seldom assessed by veterinary and public health officials per the regulations in developing countries (Okoli *et al*., 2006; Sofos, 2008; Okechukwu *et al*., 2018). However, in developing countries,local authorities which are the sole managers of slaughterhouses, have often ignored this obligation (Gali *et al*., 2020). This resulted in the deterioration of the hygienic conditions and amenities, and inadequate hygienic procedures which have negative effects on public health. As a result, There is a significant risk of pathogen detection in meat processing establishments meant for human consumption in developing countries (Ezenduka *et al*., 2010; Iroha *et al*., 2010; Unamba-Opara *et al*., 2012; Okechukwu *et al*., 2018; Bersisa, *et al*., 2019; Gali *et al*., 2020). Most slaughterhouses in developing countries especially Nigeria, animals are slaughtered on the floor and large portions of fresh meat are dipped in non-potable water for 5-20 minutes before being taken to the table for sale (eg. Nsukka and Owerri abattoirs in Nigeria) and this could affect the quality of meat for human consumption. However, this study was intended to determine the bacterial quality of beef in two regional slaughterhouses in Nigeria and antimicrobial susceptibility profile of isolates obtained.

2. MATERIALS AND METHODS

Study Area and Design

The areas of study were Owerri and Nsukka, Owerri is the capital of Imo State and located in Nigeria's south eastern rainforest vegetation zone between latitudes 5° 4^{1} and 6° 3^{1}N and longitude 6° 15^{1} and 7° 34 ^{1}E. Nsukka is in South-Eastern Nigeria, between latitude 6° 51′ 24 ^{1}N and longitude 7° 23′ 45 ^{1}E (Unamba-Opara *et al.,* 2012). The sites of the study were the municipal slaughterhouses of Owerri and Nsukka. These slaughterhouses are the primary sources of beef that is distributed and sold to the general public in both cities. This is a cross - sectional survey study that was carried out for six months (April to October, 2019).

Sampling method and sample size

The slaughterhouse at Owerri has an average daily slaughter capacity of 60 cattle, while in the Nsukka slaughterhouse, the slaughter capacity is 55 cattle. A systematic random sampling technique was used in the Owerri slaughterhouse for the experiment in which every 10th animal was selected at intervals to select 4 (four) slaughtered cattle from which the meat specimens were collected on each day of the visit. A systematic random sampling method was also used in the Nsukka slaughterhouse in which every 10th animal was selected at intervals to select 4 (four) slaughtered cattle from which the meat specimens were collected on each day of the visit.

Owerri and Nsukka municipal slaughterhouses

In Owerri municipal slaughterhousecattle were slaughtered on the floor without flaying, and the fresh chunks of meat of slaughtered cattle that were not flayed were carried on the shoulder of the butchers or wheel barrow to meat tables for sale. The same procedure took place in Nsukka municipal slaughterhouse. The only difference is that the fresh chunks of meat from slaughtered cattle were immediately put in drums that were filled with water and washed before being taken to the table for sale. The visit was done once a week in alternate weeks between Owerri and Nsukka slaughterhouses for a period of 15 weeks. During each visit to Owerri, meat samples (20g) were collected from the brisket of each of the 4 selected slaughtered cattle on the floor and from the same animals on the table, making a total of 8 meat samples. A total of 120 samples of meat were collected during 15 visits (8 x 15 visits =120). During each visit to Nsukka slaughterhouse, meat samples were collected from the brisket of each of the 4 systematically selected slaughtered cattle. Meat specimens were collected from the meat on the slaughter floor and the table from the same animal, making a total of 8 samples of the meat (Table

1). A total of 60 meat samples were collected during 15 visits (8 x 15 visits = 60). A total of 180 samples collected from these two slaughterhouses (Owerri and Nsukka slaughterhouses) were transportedtothelaboratory (microbiology lab. University of Nigeria Nsukka) in sterilized tube iniceboxes for microbial analysis.

Microbial analysis

Enumeration of the aerobic plate and total coliformcounts

Aerobic bacteria load of beef samples (aerobic plate andtotal coliform count) was determined by enumeration using the serial dilution method described by Cappuccino and Sherman (2008). On each day of visit to Owerri slaughterhouse, 25g of beef collected from each of the 4 slaughtered cattle were homogenized separately in 180 ml of sterile peptone water using a sterile stomacher bag to give a 1:10 or 10^{-1} dilution. At Nsukka slaughterhouse, 25g of beef collected from each of the 2 slaughtered cattle was ground using a sterile stomacher bag and homogenized separately in 180 mL of sterile peptone water to also give a 1:10 or 10^{-1} dilution. Serial dilutions ranging from 10^{-2} to 10^{-12} were prepared according to the recommendation of IOS (1981)

Enumeration of aerobic plate count (APC)

Enumeration of aerobic plate count was determined using the method described by Cappuccino and Sherman (2008). Aerobic plate counts (APC) were performed by dropping one-tenth (0.1mL) of each dilution (10^{-7} to 10^{-12}) onto the surfaces of well-dried nutrient agar plates with 1mL of the sterile pipette (plated out in duplicate). Each drop was uniformly dispersed using a sterilized glass spreader. Dilution of 10^{-1} to 10^{-6} was not plated or counted because of overgrowth. The plates were incubated aerobically at 30 ºC for 72 hours. The colony-forming units per gram (cfc/g) of beef were calculated by counting the growing colonies with a colony counter.

Enumerationof total coliform count(TCC)

Enumeration of the total coliform count was determinedusing the method described by Cappuccino and Sherman (2008). For total coliform bacteria counts, 0.1ml of each dilution (10^{-7} to 10^{-12}) were dropped onto the surfaces of a well-dried sterile MacConkey agar plates using a 1mL sterile pipette (plated out in duplicate). A sterile glass spreader was used to distribute the drop uniformly. Dilution of 10^{-1} to 10^{-6} was not plated or counted because of overgrowth. The plates were incubated aerobically at 30 ºC for 72hr. Pink colonies, (lactose positive) were counted and recorded as colony-forming units/gram of meat (cfc/g) using a colony counter.

The colony-forming units (ufc) per gram of undiluted meat were calculated and recorded using a colony counter for both the viable count and the coliform count. The number of colonies in plates containing between 30-300 colonies was considered good and adequately reported. Plates with less than 30 colonies were considered too few to count (TFTC), whereas plates with more than 300 colonies were considered too numerous to count (TNTC). Both TFTC and TNTC plates were rejected without being recorded.

Table 1: Breakdown of sample collection and size

	Owerri			**Nsukka**		
	Sample collection/visit			**Sample collection/visit**		
Location of Meat	**Samples on each visit**	**No. of visit**	**Total**	**Samples on each visit**	**No. of visit**	**Total**
Floor	4		60	4		60
Table	4	15	60	4	15	60
Total	**8**		**120**	**8**		**120**

The load of the respective aerobic bacterial types from each sample was calculated using the formula described by Cappucino and Sherma (2008) as shown

$$C = n/vd$$

Where C = colony forming unit per gram (ufc/g)
n = number of colonies
d = dilution factor
v = volume transferred to plate = 1

The result was recorded as ufc/g. The mean counts for each of the meat samples from slaughtered cattle from the 2 slaughterhouses were taken.

Determination of the prevalence of Salmonella and E. coli in beef samples;

Isolation of Salmonella

This was done in accordance with the International Commission on Microbiological Specifications for Foods' method (ICMSF, 1996). For pre-enrichment, a piece of each meat sample was inoculated into 10 mL buffered peptone water. The inoculated samples were inoculated for 24 hours at 37 oC. Following a 24-hour incubation period, 0.1 mL of the pre-enriched broth was inoculated in 1 mL of Rappaport Vassiliadis (RV) medium for selective enrichment using a 1 mL pipette. After inoculating the RV broths, they were incubated at 24 oC for 48 hours. A loopful of the enrichment broth was plated onto MacConkey and Brilliant Green agar after 24 hours. The inoculated plates were incubated for 24 hours and observed for colonial characteristics.

On MacConkey, typical *Salmonella* produce colourless (non-lactose fermenting) whereas on brillant green agar, *Salmonella* produced red-pink colonies with surrounding brilliant red zones after incubating the plates for 24 hours at 37 ºC. Suspected *Salmonella* isolates were gram stained using Cuppaccino and Sherman (2008) technique, and examined under the microscope for gram-negative short rods. The suspects were inoculated on nutrient agar (NA) slants, incubated at 37 ºC for 24 hours and then stored at 4 ºC for 24 hours for further identification.

Isolation of E. coli

Isolation of *E. coli* was done by the method described by the International Commission on Microbiological Specifications for Foods (ICMSF, 1996). Each of the samples (meat specimen) was streaked on MacConkey agar. The inoculated plates were explicitly labeled and incubated at 37 ºC for 24 hours. Pink colonies were identified and sub-cultured on fresh MacConkey agar (MCA) for purification. The colonies were then gram-stained and observed for gram negative (pink) medium sized slender rods. Suspected colonies were further sub-cultured on Eosin methylene blue (EMB) agar, incubated at 37 ºC for 24 hours and examined for the presence of a greenish metallic sheen (typical of *E. coli*).

For biochemical identification and confirmation (Indole, methyl - red, voges – proskauer, simmon's citrate, urease and triple sugar iron agar tests) of *Salmonellaand E. coli* isolates, stocked cultures of *Salmonella* and *E. coli* suspects were sub-cultured on MCA, incubated at 37 ºC for 24 hours and used for the biochemical tests. Biochemical identification and confirmation were carried out by the method described by Cuppacino and Sherman (2008). Verotoxygenic *E. coli* (VTEC) contamination of beef in Nsukka and Owerri slaughterhouses was also determined.

Serological detection of O157 VTEC strains

All *E. coli* strains isolated were sub-cultured on the cultured agar plate plates, incubated at 37 ºC for 24 hours and used for the test. The dry spot *E. coli* serocheck was brought out from the refrigerator and allowed to attain room temperature on a bench before opening it. This is to ascertain whether the serocheck kit was working properly before use, a drop (50ul) of the negative control was mixed with a drop of the dry latex reagent and this should produce no agglutination within 60 seconds. Fifty micro-liter of phosphate-buffered saline was dropped onto a small ring (at the bottom of each ring) in both the test and control reaction areas ensuring that the liquid does not mix with the dry latex reagents. Using a sterile loop 2 mm diameter colonies from a cultured agar plate were picked and applied to the control reaction area (using 2 or more colonies if they are smaller than 1mm). The colonies were emulsified in phosphate-buffered saline to obtain a slightly turbid suspension. Each suspension was mixed onto

dried latex spots until completely suspended and spread to cover the reaction area. The paddles were discarded appropriately into a disinfectant jar. The card was picked and rocked for up to 60 seconds and checked for agglutination under normal lighting condition. When the test was done completely, the reaction card was disposed safely into disinfectant. A positive reaction is observed when agglutination was indicated by formation of clumps in the reaction areas within 60 seconds

Serological test for non-O157 VTEC strains

The sorbitol positive isolates (non-O157 VTEC strains) were inoculated onto nutrient agar slants in bijou bottles and stored at the temperature of 8 ºC for further characterization. Sorbitol positive isolates (non-0157) were subjected to polyvalent serocheck test kits containing six serotypes O26, O45, O103, O111, O121 and O145 from Oxoid Ltd, Hampshire, England.

Determination of the antimicrobial resistance profile of E. coli and Salmonella isolates

The antibiogram of the isolates was conducted using the disc diffusion method of the Clinical Laboratory Standard Institute (CLSI, 2010). The 10 antimicrobial agents used were: neomycin (30 ug), doxycycline hydrochloride (30 ug), amoxycillin-clauvulanic acid (30 ug), gentamicin (30 ug), tetracycline (30 ug), amoxycillin (10 ug), sulphamethoxazole /trimethoprim (25 ug), nitrofurantoin (300 ug), ciprofloxacin (5 ug), streptomycin (10 ug). Product of Oxoid® UK. A single colony (2×10^8ufc/mL) of each of the test organisms (tested on 0.5 mcfarland standard to standardize the approximate number of bacteria in a liquid suspension) was picked with a sterile wire loop and inoculated onto 3mL of sterile nutrient broth. The inoculated nutrient broth was incubated for 1 hour, after which sterile colonies of a pure culture of the isolates were grown on Mueller Hinton medium using the pour plate method. By the use of an aseptic technique, antibiotic discs were placed onto the inoculated Mueller Hinton agar. The plates were then incubated at 35 ˚C for 24 hours. Each isolate was tested in duplicate. After incubation, the diameter of the inhibition zone produced around each disc was measured with a meter rule and the average inhibition zone diameter was recorded to the nearest whole millimeter. Susceptibility to the discs was observed as a clear zone of inhibition around the discs. The inhibition zone diameters (IZDs) were measured and recorded as resistance, intermediate and sensitive following the CLSI (2010) guidelines.

Data presentation and analysis

The mean of the total viable bacteria and*coliform counts* were determined and statistically analysed by students t-test using SPSS version 20.0. Chi-square statistical analysis ($p<0.05$) was used to determine any association between the prevalence of isolated microorganisms and the locations (Nsukka or Owerri) of abattoirs.

3. RESULTS AND DISCUSSION

Aerobic plate and coliform count of meat from the floor and table

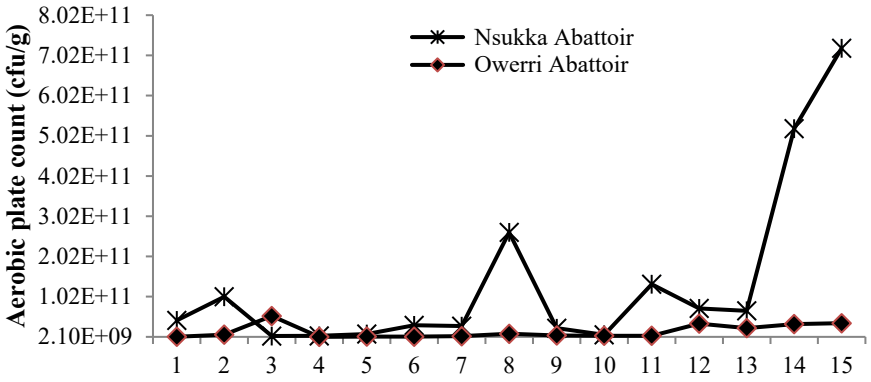
Aerobic plate count

The values in Figure 1 show the bacterial quality of beef slaughtered in Nsukka and Owerri abattoirs. The total aerobic plate count of meat slaughtered on the floor ranges from 4.3×10^9 to 7.2×10^{11} cfu/g in the Nsukka abattoir, and this increased significantly ($p<0.05$) when displayed on the table for sale after water immersion (1.05×10^{11} to 9.9×10^{12} cfu/g). The aerobic plate count of meat collected on the floor of the Owerri abattoir ranges from 2.10×10^9 to 5.4×10^{10} cfu/g while meat displayed on the table ranges from 1.12×10^{10} to 8.4×10^{11} cfu/g. The mean value of the aerobic plate count of meat collected from Nsukka abattoir floor (1.3×10^{11} cfu/g) was significantly ($p < 0.05$) higher than that of the meat collected from the Owerri (1.42×10^{10} cfu/g) abattoir floor. The mean aerobic plate count of meat from the Owerri abattoir table (2.9×10^{11} cfu/g) was significantly ($p<0.05$) higher than that of the meat from the Nsukka abattoir table (4.6×10^{10} cfu/g). The high overall number of bacteria found on the abattoir floor and table exceeded the acceptance level ($> 10^6$) (WHO, 2007), which indicates that the level of hygiene work. Because the bacterial count in fresh meat exceeds the above standard, the meat is unsafe

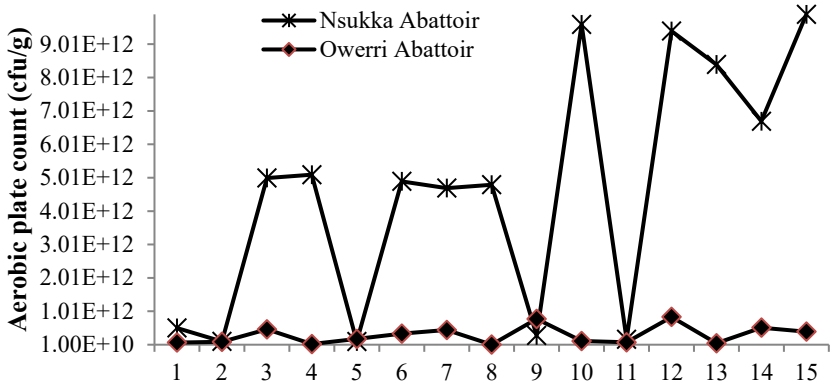
to eat, raising concerns about meat quality from the abattoir to butcher shops (Chuku *et al*. 2016). Significantly ($p < 0.05$) higher aerobic plate count of meat from Nsukka abattoir floor than Owerri abattoir floor could be due to the fact that animals were flayed on the floor during the slaughter in the Nsukka abattoir, which was not done in the Owerri abattoir. Similar results were reported by Chuku *et al*. (2016) (Lafia Metropolis, Nigeria), Haileselassie *et al*. (2013) (Ethiopia's Mekelle abattoir), and Bersisa *et al*. (2019) (abattoir and butcher Shops in Bishoftu, Central Ethiopia).

Total coliform count

The result in Figure 2 also shows that thetotal coliform count of meat slaughtered on the floor ranges from 1.2×10^8 to 1.8×10^{10} cfu/g which increased significantly ($p<0.05$) when displayed on the table for sale after immersion in water (2.5×10^8 to 5.6×10^{10} cfu/g). The total coliform count of the meat collected on the floor in the Owerriabattoir also ranges from 1.4×10^8 to 7.1×10^9 cfu/g and from 1.4×10^9 to 2.6×10^{10} cfu/g for meat on the table. Though there was no significant difference in the mean total coliform count between meat from Nsukka and Owerri (floor) abattoirs. The overall coliform count of meat also exceeded the above the acceptable range ($>10^6$) (WHO, 2007) and this could be due to animals being slaughtered on the floor without being flayed and fresh pieces of meat being transferred from the slaughtered cattle to the table using a wheel barrow or the butcher's shoulder. Similar results were reported by Ezenduka *et al*. (2010), Adetunji and Odetokun (2011) and Haileselassie *et al*. (2013). Ezenduka *et al*. (2010) identified wheelbarrows and immersion water as potential sources of contamination of market meat.

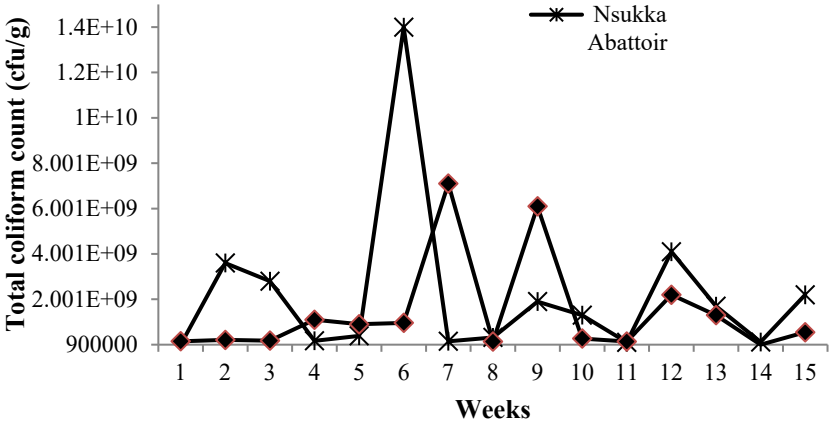


1a: Aerobic plate count of meat from the floor

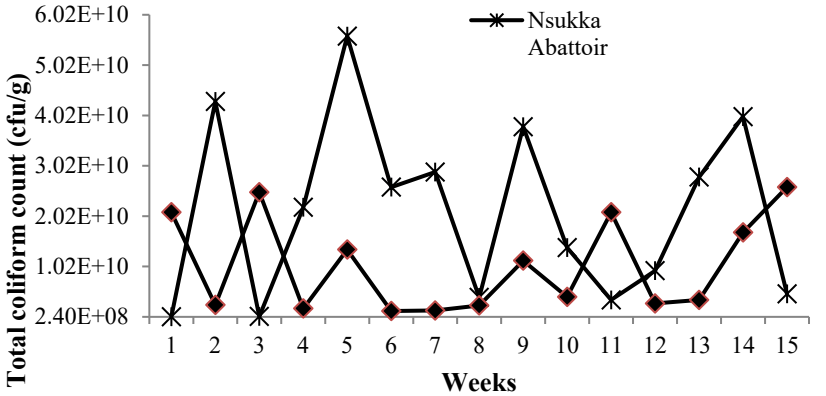


1b: Aerobic plate count of meat from the table

Figure 1: Aerobic platecounts of meatfrom the floor and table



2a: Total coliform count of meat from the floor



2b: Total coliform count of meat from the table

Figure 2: Total coliform counts of meat from the floor and table

Prevalence of E. coli and Salmonella in meat samples

Prevalence of E. coli

E. coli Prevalence

Table 2 shows the prevalence of *E. coli* and *Salmonella* in meat collected from the floor and tables in the abattoirs of Nsukka and Owerri. In Nsukka abattoir, out of 60 meat samples collected on the floor after slaughter, 27 (45 %) were positive for *E. Coli*, while out of 60 meat samples collected on the table after immersion in water, 38 (63.%) were positive for *E. coli*. The 27 and 38 confirmed *E. coli* positive samples collected on the floor after slaughter and on the table after water immersion were all grown on Sorbito Mcconkey (SMAC), and all of them produced positive pink colonies indicating non-O157. The 27 and 38 non O157 isolates were further tested for Serocheck test containing six serotypes: O111, O45, O103, O26, O145, and O121. Eleven (41 %) of the 33 meat samples collected on the floor after slaughter were positive for Serocheck. Sixteen (42%) of the 38 meat samples collected on the table after water immersion were also positive for Serocheck (i.e., belonging to one or more of the 6 Serotypes). Out of 60 meat samples collected from the floor in Owerri abattoir, 18 (30%) were positive for *E. Coli,* while out of the same 60 meat samples collected from the table (meat for sale), 31 (51%) were positive for *E. coli*. The 18 (30%) and 31 (51.7%) confirmed *E. coli* positive samples collected on the floor after slaughter and on the table were grown on SMAC and all of them produced positive pink colonies indicating non-O157. The 18 and 31 non O157 isolates were further tested for serocheck test containing six serotypes: O111, O45, O103,

O26, O145, and O121. Six (33.3%) out of the 18 meat samples collected from the floor were positive for serocheck, as shown in Table 3. Eleven (35.5%) out of the 31 meat samples collected from the table were also positive in the serocheck (i.e belonging to one or more of the 6 serotypes). The prevalence of *E. coli* in meat samples from the Nsukka abattoir (45%) was higher than that of the Owerri abattoir (30%). In Nsukka abattoir, it was found that more meat samples from the table after immersion in water were positive for *E. coli* than meat on the floor after slaughter. This could be due to butchers' use of water to wash meat (immediately after slaughter) without replacement, which could lead to an increase in the bacterial load of the water. A similar result was reported by Ezenduka *et al*. (2010) on the open transportation of carcasses/bulk meat on wheelbarrows from the abattoir to the sales joint. From the contaminated meat, *Bacillus sp., Escherichia coli, Klebsiella sp., Pasteurella sp., Staphylococcus sp., Streptococcus sp.,* and *Salmonella sp*. were isolated as pathogens by Ezenduka *et al*. (2010).

Table 2: Prevalence of *E. coli* and *Salmonella* in meat

Microorganisms			Meat on the flour		Meat on the table	
			Nsukka abattoir	Owerri abattoir	Nsukka abattoir	Owerri abattoir
E. coli		Positive	27 (45%)	18 (30%)	38 (63%)	31 (51%)
		Negative	33 (55%)	42 (70%)	22 (37%)	29 (49%)
		Total	**60** (100%)	**60 (100%)**	**60 (100%)**	**60 (100%)**
***E. coli* (Positive)**	SMAC (non 0157)	Positive (pink)	27	18	38	31
		Negative (colourless)	0	0	0	0
	Serocheck (seroscreen)	Positive	11 (41%)	6 (33%)	16 (42%)	11 (35%)
		Negative	16 (59%)	12 (66%)	22 (58%)	20 (65%)
Salmonella		**Positive**	20 (33%)	3 (5%)	25 (41%)	4 (7%)
		Negative	40 (67%)	57 (95%)	35 (59%)	56 (93%)

Table 3: Antimicrobial susceptibility profile of *E. coli* and *Salmonella*

Antimicrobial agent	Con. (µg)	Number (Percentage) of isolate = n							
		Nsukka (n = 35 for *E. coli*, n =15 for *Salmonella*)				Owerri (n = 49 for *E. coli*, n = 7 for *Salmonella*)			
		Resistant		Susceptible		Resistant		Susceptible	
		E. coli	*Salmonella*	*E. coli*	*Salmonella*	*E. coli*	*Salmonella*	*E. coli*	*Salmonella*
Neomycin	30	20 (57.15)	6 (40)	15 (42.86)	9 (60)	26 (53.06)	1 (14.29)	23 (46.94)	6(85.71)
Gentamicin	30	5 (14.29)	3 (20)	30 (85.71)	12 (80)	3 (6.12)	0 (0)	46 (93.88)	7(100)
Amoxycillin	10	35 (100)	15 (100)	0 (0)	0 (0)	49 (100)	4 (66.6)	0 (0)	3(33.30)
Tetracycline	30	15 (42.86	6 (40)	20 (57.14)	9 (60)	41 (83.67)	4 (66.60)	8 (16.33)	3(33.30)
Doxycycline	30	5 (14.29)	0 (0)	30 (85.71)	15 (100)	30 (61.22)	0 (0)	19 (38.78)	7(100)
Nitrofuratoin	30	15 (42.86)	6 (40)	20 (57.14)	9 (60)	11 (22.45)	3 (42.86)	38 (77.55)	4(57.14)
Streptomycin	10	35 (100)	15 (100)	0 (0)	0 (0)	41 (83.67)	5 (71.43)	8 (16.33)	2(28.57)
Amoxicillin-Clauvulanic acid	30	35 (100)	0 (0)	0 (0)	15 (100)	46 (93.88)	7 (100)	3 (6.12)	0(0)
Ciprofloxacin	5	10 (28.58)	3 (20)	25 (71.43)	12 (80)	19 (38.32)	2 (28.58)	30 (61.22)	5(71.43)
Sulphamethoxazole /Trimethoprin	25	10 (28.57)	3 (20)	25 (71.43)	12 (80)	23 (46.94)	1 (14.29)	26 (53.06)	6(85.71)

Values in the bracket are in percentage.

A higher prevalence of *E. coli* was associated with meat samples from the table. The result of the present study was higher than that reported by Adetunji and Odetokun (2011), research conducted in the Ibadan abattoir in the Southwest of Nigeria. The prevalence of *E. coli* in meat on the flour and table in Nsukka and Owerri abattoirs indicates the presence of fecal contamination from animals and humans (Iroh *et al*., 2010). A comparable result was reported by Ezenduka *et al*. (2010). Ezenduka *et al*. (2010) also revealed that transporting the chunks of meat on the dirty butchers' shoulders and unclean wheel barrows to display on the table for sale could be a source of bacterial contamination of the meat, which is in violation of National Environmental Health Practice Regulations (2016). The prevalence of *E. coli* in meat samples from the Nsukka abattoir was higher than that of the Owerri abattoir, which may be due to poor handling and the use of non-portable water during meat immersion and washing after slaughter, both of which are in violation of the National Environmental Health Practice Regulations (Ezenduka *et al*., 2010; NEHPR 2016). The rise in contamination could also point to noncompliance with FAO standards for slaughterhouse design and operation (FAO, 1988).

Prevalence of Salmonella

In the Nsukka abattoir, out of 60 meat samples collected on the floor 20 (33%) were positive for *Salmonella* while 25 (41%) were positive out of 60 meat samples collected on the table after immersion in water (Table 3). Comparable results were reported by Iroha *et al*. (2010) on raw meat sold in Abakaliki, Ebonyi State Nigeria in which out of the 300 samples, 79 (29.3%) were contaminated with bacteria species including *Salmonella typhi*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Shigella dysenteriae* and *Staphylococcus aureus* which is similar to the report of Ezenduka *et al*. (2010).Washing of meat with non-potable water could lead to an increase in *salmonella* in meat (Iroha *et al*., 2010). In the Owerri abattoir out of 60 meat samples collected on the floor, 3 (5%) were positive for *Salmonella* while out of 60 meat samples collected from the table (for sale), 4 (6.6%) were positive for *Salmonella*. In the Owerri slaughterhouse, 5% and 7% of the meat samples from the floor and table contained *Salmonella*,respectively. A similar result was reported by Iroha *et al*. (2010) and Ezenduka *et al*. (2010).

Antimicrobial susceptibility profile of E. coli and Salmonella

Antimicrobial susceptibility profile of E. coli

The result in Table 3 shows that thirty-five (35) *E. coli* strains isolated from beef samples in the Nsukka abattoir exhibited the highest resistance to amoxicillin, streptomycin and amoxicillin-clauvalanic acid (100% each);Twenty (57.14%) were resistant to neomycin; fifteen (42.86%) were resistant to tetracycline and nitofuratoin, ten (28.57%) were resistant to sulphamethoxazole/trimethoprim and ciprofloxacin while five (14.29%) were the least resistant to doxycycline and gentamicin. In the slaughterhouse of Owerri, 49 *E. coli* strains isolated showed the highest resistance to amoxicillin (100%); forty-six (93.88%) were resistant to amoxicillin-clauvalanic acid; forty-one (83.67%) were resistant to tetracycline and streptomycin; thirty (61.22%) were resistant to doxycycline; twenty six (53.06%) were resistant to neomycin; twenty-three (46.94%) were resistant to sulphamethoxazole/trimethoprim; nineteen (38.77%) were resistant to ciprofloxacin; eleven (22.45%) were resistant to nitrofuratoin and the least resistance to gentamicin 3(6.12%). The same result was reported by Iroha *et al*., (2010) on raw meat sold in Abakaliki, Ebonyi State Nigeria which the susceptibility results of bacteria isolated from meat samples showed that they are highly resistant to all the antibiotics tested (ciprofloxacin, amoxicillin, ampicillin, gentamicin, cephalexin, cotrimoxazole, clindamycin, erythromycin) but contract to the reported of Unamba-Opara *et al*. (2012) on detection of verotoxigenic *Escherichia coli* (VTEC) from cattle slaughtered at Nsukka Abattoir, Nigeria. Unamba-Opara *et al*. (2012) reported that of all the sorbitol positive *E. coli*, 50 strains were randomly selected and tested with the Serocheck kit, two strains (4%) were

found to belong to the VTEC group. All the antibiotics used in the test were effective against the two strains. Although Unamba-Opara *et al*. (2012) claimed that the number of strains examined was insufficient to provide a meaningful sensitivity profile to be made. Iroha *et al*. (2010) also reported that gram-negative organisms are more resistant than the Gram-positives. This is expected because of the intrinsic nature of the gram-negative cell wall.. Because of the inherent nature of the gram-negative cell wall, this is to be anticipated. Antibiotic use in animal breeding and treatment of bacterial illnesses in animals might be responsible for the reported resistance. Antibiotic-resistant bacteria that can be transmitted to humans through meat consumption and pose a public health risk (Iroha *et al*., 2010).

Antimicrobial susceptibility profile of Salmonella

The result in Table 3 also shows that fifteen (15) *Salmonella* isolates (100%) in Nsukka abattoir were resistant to amoxicillin and streptomycin; six (40%) were resistant to tetracycline, neomycin and nitrofuratoin; three (20%) were resistant to sulphamethoxazole/trimethoprim, ciprofloxacin and gentamicin and had minimal resistance to amocillin-clauvalanic acid and doxycycline. Seven (7) *Salmonella* isolates (100%) were resistant to amoxicillin-clauvalanic acid; five (71.43%) were resistant to streptomycin; four (66.60%) were resistant to tetracycline and amoxicillin; three (42.86%) were resistant to nitrofuratoin; two (28.58%) were resistant to ciprofloxacin; one (14.29%) was resistant to neomycin and sulphamethoxazole/trimethoprim while none (0%) of the isolates was resistant to doxycycline and gentamicin. The *Salmonella* isolates showed the lowest (0%) resistance to doxycycline and amoxicillin clauvulanic acid in Nsukka abattoir and 0% resistance to gentamicin and doxycycline in Owerri abattoir. This means that *salmonella* isolates are susceptible to doxycycline and gentamicin. A similar result was reported by Ejo *et al*. (2016) who isolated *Salmonella* from animal-origin food items in Gondar, Ethiopia; among *Salmonella* isolates tested, 42.6%, 28.6%, and 14.3% were found to be relatively resistant to tetracycline, sulfamethoxazole-trimethoprim, and ampicillin, respectively, while 9.5% - 19% were intermediately resistant to tetracycline, amoxicillin, ampicillin, cephalothin, and nitrofurantoin. The rise of antimicrobial-resistant *salmonella* is linked with the use of antibiotics in animals raised for food; resistant bacteria can be transfered to humans via foods, particularly those of animal origin (White *et al*., 2001)

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