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Occurrence of multidrug-resistant *Salmonella* in cattle carcass and contact surfaces in Kwata slaughterhouse, Awka, Anambra State, Nigeria

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Abstract

Background and Aim: Contamination of meat with *Salmonella* could result in food-borne disease outbreaks among the consumers. However, there is a dearth of data assessing the presence of *Salmonella* in beef in Anambra State. Therefore, this study determined the prevalence and antimicrobial susceptibility of *Salmonella* in beef and slaughter/processing facilities in Kwata slaughterhouse.

Materials and Methods: Swab samples (200) randomly collected were cultured for the isolation of *Salmonella* and the isolates subjected to antimicrobial susceptibility test. Data obtained were analyzed using t-test and analysis of variance with $p< 0.05$ considered statistically significant.

Results: Of the 200 samples cultured, 33.5% (67/200) yielded *Salmonella* isolates. The mean *Salmonella* load (colony-forming unit [CFU]/cm^2) for different contact surfaces (before and after contact with carcasses) was as follows: Slaughter floor, $1.1\times10^{10}\pm1.1\times10^{6a}$ and $1.0\times10^{10}\pm1.1\times10^{6b}$; display table, $1.1\times10^{10}\pm11.1\times10^{6a}$ and $1.0\times10^{10}\pm1.1\times10^{6b}$; washing bucket $1.01\times10^{10}\pm1.0\times10^{6a}$ and $0.8\times10^{10}\pm0.1\times10^{6b}$; knife, $1.1\times10^{10}\pm1.10\times10^{6a}$ and $1\times10^{10}\pm1.0\times10^{6b}$; boot, $1.1\times10^{10}\pm1.0\times10^{6a}$ and $1.0\times10^{10}\pm1.10\times10^{6b}$; file, $1.1\times10^{10}\pm1.0\times10^{6a}$ and $1.0\times10^{10}\pm0.1\times10^{6b}$; and wheelbarrow, $1.1\times10^{10}\pm1.0\times10^{6a}$ and $1.01\times10^{10}\pm0.11\times10^{6b}$. *Salmonella* counts decreased significantly ($p<0.05$) in the presented order from slaughter floor to wheelbarrow after contact with carcasses. On the other hand, there was a significant ($p<0.05$) increase in washing water *Salmonella* counts before and after ($0.7\times10^{10}\pm0.10\times10^{6a}$ and $1.0\times10^{10}\pm1.0\times10^{6b}$ CFU/100 ml) carcasses wash. To each of the antimicrobials tested, the percentage of the 67 isolates found resistant was as follows: ciprofloxacin, 25.4%; ofloxacin, 27%; ceftriaxone, 35.8%; amoxicillin/clavulanic acid, 88.1%; chloramphenicol, 59.7%; gentamicin, 34.3%; streptomycin, 49.3%; nalidixic acid, 49.3%; trimethoprim/sulfamethoxazole, 76%; nitrofurantoin, 89.6%; and ampicillin, 100%.

Conclusion: Antimicrobial-resistant *Salmonella* were isolated from beef and slaughter/processing facilities in Anambra State. This underscores the need for a coordinated one health approach for the improvement of hygienic standard during slaughter/processing in the slaughterhouse surveyed, to limit meat contamination and hence safeguard human health.

Keywords: beef, contact surfaces, Kwata slaughterhouse, resistance, *Salmonella.*

Introduction

Food-borne pathogens are known to cause illnesses and deaths as well as enormous amounts of money in medical care and social costs globally. Consumption of contaminated meat has been associated with outbreaks of food-borne diseases. Quite a good number of food-borne bacterial infections of animal origin are attributed to contamination of animal carcasses during slaughter and processing [1]. This can be averted following proper hygiene slaughter practices. In Nigeria, beef consumed by the public is usually sourced from slaughterhouses where the law stipulates hygiene standards as well as ante- and post-mortem inspections of slaughter animals to reduce contamination. Unfortunately, poor hygiene practices result in abattoirs becoming sources of meat contamination. The amount of bacterial contamination of carcasses in slaughterhouse is affected by sanitation levels during farm management procedures, transport of animals to the abattoir, keeping of animals awaiting slaughter at the lairage, slaughter, and processing in the abattoir as well as retail and final processing before consumption [2]. Top among hygiene slaughter challenges in developing countries, including Nigeria, is the lack of necessary infrastructure that enhances hygiene processing such as potable water supply, drainage, and proper waste disposal facilities [3]. This is exacerbated by poor knowledge, unhygienic methods of slaughter, and processing activities among slaughterhouse personnel as well as government's lack of political will to enforce hygiene slaughter and meat inspection laws in

slaughterhouses [4,5]. In most Nigeria slaughterhouses, activities such as slaughter, flaying, carcass splitting, and intestinal evisceration are usually performed on the floor resulting in profound contamination of the floor and carcasses with feces [5-7]. Livestock feces are heavily populated with Enterobacteriaceae, which are commensal flora of the gastrointestinal tract [8]. They are always present in the slaughterhouse environment, and being harbored and shed by animals and slaughterhouse personnel, are the most common contaminating aerobic bacteria in slaughterhouses [9-11]. The Enterobacteriaceae are responsible for multiple disease outbreaks all over the world, leaving misery and financial losses in their trail [12-14].

A member of the Enterobacteriaceae family, *Salmonella*, has been described as one of the leading causes of food-borne diseases worldwide [15]. The members of the species are the most common causes of septicemia in children and the elderly resulting in high morbidity and mortality [16]. According to Stanaway *et al*. [17], there were about 14.3 million typhoid and paratyphoid cases resulting in estimated 135.9 thousand deaths with higher case fatality estimates among children, older adults, and among lower-income countries as well as being responsible for 9.8 million disability-adjusted life-years in 2017, globally. Food-borne infections due to *Salmonella* spp. are mainly due to the consumption of foods of animal origin contaminated with feces [18,19]. This is worse in a developing country like Nigeria where public health laws are not stringently enforced. Although salmonellosis is associated with food-borne transmission, recent contact with cattle has also been associated with the disease in humans [20]. Therefore, slaughterhouse personnel are at high risk of infection through regular contact with slaughter animals.

The emergence of antimicrobial-resistant *Salmonella* recovered from meat products has become a source of major concern. Pathogens are reported to inflict more serious damage when they are antimicrobial-resistant [21]. Resistance results in longer stay in hospital as well as increased cost and less effective chemotherapy that may become toxic to the patient. Infections due to resistant *Salmonella* are reported to result in increase in morbidity and mortality, especially in immune-compromised patients [18]. Obviously, the presence of resistant *Salmonella* in meat is a threat to public health. *Salmonellae* resist drugs through enzymatic degradation, alteration of site of actions, blocking cell permeability, efflux pumps, and horizontal transfer of resistant genes [18,22,23]. Drug-resistant *Salmonella* are capable of transmitting antibacterial resistance genes to other bacteria, thereby maintaining the dissemination of antimicrobial resistance among bacteria in the food chain [24]. In Nigeria, multidrug-resistant *Salmonella* spp. have been isolated from various sources: Water [25], farm produce and environment [26], abattoir environment [27], dogs [28], poultry [29], and humans [30,31]. Therefore, the isolation and determination of antibiogram of *Salmonella* isolates from the slaughterhouse environment and the meat processed within it is crucial for devising strategies and policies that ensure provision of wholesome and safe meat for the protection of consumers and reduction of antibiotic resistance in Nigeria [1,24]. Although several works have been conducted on the hygiene levels of slaughterhouses, information on *Salmonella* contamination of the cattle carcass (the edible part mostly consumed) is scanty. In addition, data in available literature on antimicrobial resistance profiles of *Salmonella* isolates from slaughterhouses are lacking in Anambra State.

This work was, therefore, conducted to isolate and assess the antibiotic resistance patterns of *Salmonella* in meat and the contact surfaces in Kwata slaughterhouse, Awka, Anambra State, Southeast, Nigeria.

Materials and Methods

Ethical approval

The ethical permission for this work was obtained from the University of Ibadan Animal Care and Use Research Ethics Committee (No. UI-ACUREC/APP/2015/047) and the research was conducted in accordance with the guidelines laid by the committee as well as local laws and regulations.

Study period and study area

The study was conducted from May to November, 2018 in Kwata slaughterhouse, Awka, Anambra State, Southeast Nigeria. Awka is located at latitude 8.2069°N and longitude 7.0678°E (Figure-1) and has a population of 361,657 (Nigeria Population Commission, 2006). Awka is noted for her iron craft and wood carving although becoming the capital of Anambra State in 2001 brought about the influx of civil servants to the town. With the increase in population, few nomadic herds have settled at the outskirts of the town. The slaughterhouse supplies the bulk of the beef consumed in the town which originate from cattle sourced from local herds, northern parts of Nigeria and neighboring countries.

Study design

The cross-sectional study design was used for this study.

Sample size determination

The sample size for bacteriological examination was determined according to the formula described by Aroaye [32] using the average prevalence of 9.46% (from 10.00% [33] and 8.92% [34]), the minimum sample size calculated was n=138 samples. However, 200 samples were used for the study to improve the accuracy of the data.

Sampling method, sample collection, transportation, and processing

The slaughtering and processing points in Kwata slaughterhouse were visited for sampling once weekly for 10 weeks between May and November.

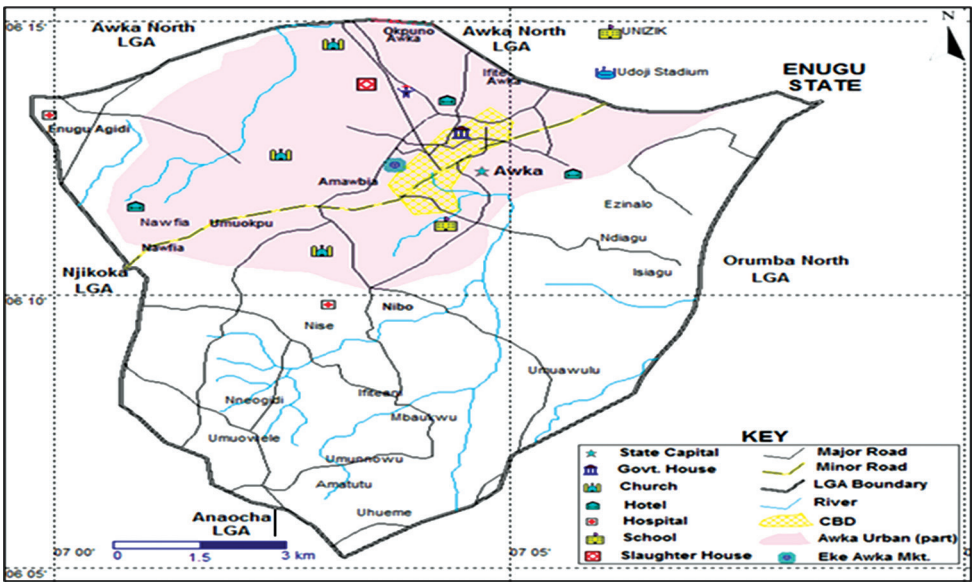

Figure-1: Map of Awka metropolis (Source: Department of Geography, University of Nigeria, Nsukka).

A systematic random sampling of 1:5 was used to select 20 carcasses from which samples were collected each week. Using the list of the workers in the abattoir as the sampling frame, balloting was used to collect samples from portions of contact surfaces (washing bucket [WBU], wheelbarrow, sharpening file, knife, workers' boot, display table, and surface of beef carcasses). As for the slaughter slab (SS), the center was identified with measuring tape and a protractor was used to mark out 36°C along which samples were collected in clockwise direction weekly. Furthermore, water (5 ml) used for washing beef carcasses was collected from the tap and drainage channel during slaughtering as well as before and after processing. Swab samples were collected from 2 cm^2 of each of the contact surfaces using sterile swab sticks moistened with 0.1% sterile peptone water and a 2 cm^2 template before and after contact with beef carcass as follows: SS, dressing table, WBU, meat cutting knife, slaughterhouse workers' boot before and after, wheelbarrow, and knife sharpening file before and after contact with knife used for cutting meat as well as beef carcass surface before and after contact with the SS. Beef (10 g) was collected before and after contact with the SS and suspended in 0.1% sterile buffered peptone water contained in sterile half ounce bottles. Water (10 ml) used for beef carcass washing was also collected aseptically before and after washing of beef carcass using sterile syringes. A total of 200 samples made up of 20 samples (each of weekly visits) for 10 weeks from meat, water, and swabs of cattle carcasses, contact surfaces during processing and transportation of beef were collected (Table-1). The samples were transported aseptically in ice packs and processed within 12 h of collection in the laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Nigeria, Nsukka.

Determination of bacterial load of the contact surfaces sampled

The *Salmonella* load of the samples were determined by enumeration using serial dilution [35] and the mean counts recorded in colony-forming unit (CFU)/cm^2.

Isolation and identification of *Salmonella*

Isolation and identification of *Salmonella* was done following the protocol described by the International Commission on Microbiological Specifications for Foods [36].

Antibiogram of the isolates

Antimicrobial susceptibility of the bacteria isolates was determined by the disk diffusion method as described by the Clinical and Laboratory Standards Institute [37].

Statistical analysis

Data obtained for the prevalence and the antibiogram were expressed in percentages, while that obtained from bacteria counts were summarized as mean values. The mean values of the bacteria counts were compared using one-way analysis of variance in Statistical Package for the Social Sciences version 15.0 (SPSS, IBM Inc., NY, USA). Duncan's multiple range test was used to separate variant means and p-values less than 0.05 were considered significant.

Results

Out of the 200 samples cultured, a total of 67 (33.5%) *Salmonella* isolates were obtained (Table-1). *Salmonella* load counts decreased significantly ($p<0.05$) after contact with the carcasses in the following order: Slaughter floor, display table, WBU, knife, boot, file, and wheelbarrow after contact with beef carcasses. There was a significant ($p<0.05$) increase in the counts of *Salmonella* in water after its use in washing of the beef carcasses. In addition, mean *Salmonella* loads of

the carcasses surfaces increased from $1.0\times10^{10}\pm1.0\times10^{6a}$ to $1.31x10^{10}\pm1.01\times10^{6b}$ CFU/cm^2 before and after contact with the slaughter floor. Moreover, mean *Salmonella* loads of beef carcasses also increased from $1.0\times10^{10}\pm1.10\times10^{6a}$ CFU/cm^2 before processing to $1.10\times10^{10}\pm10\times10^{6b}$ CFU/cm^2, after processing. There were no significant ($p>0.05$) increases in the mean counts of *Salmonella* in the cattle carcasses after contact with the slaughter floor and after processing (Table-2).

The antibiogram showed that a range of 8 to 100% of the 67 *Salmonella* isolates, were resistant to the 11 antimicrobials tested (Table-3).

Discussion

The study isolated multidrug-resistant *Salmonella* from cattle carcasses after slaughter and during processing activities in KSH. This is of public health and economic importance in Nigeria where the incidence of gastroenteritis, enteric fever, and bacteremia in children is on the increase [31,38]. Resistant *Salmonella* species has been noted to affect the quality of clinical management and rate of success in the treatment of salmonellosis in humans and animals, resulting in increased morbidity and mortality [31]. The observed resistance of the isolates could be attributed to the frequent imprudent use of antibiotics in food animals in Nigeria [39]. The abuse of antibiotics is widespread in the country given that prescription drugs of all classes are sold over the counter and used without proper diagnosis, prescription, or professional oversight [40]. Such unsupervised and indiscriminate use of antibiotics is noted to exert selection pressure on microorganisms, resulting in drug resistance [41]. Furthermore, the resistance observed in the isolates poses a risk to consumers of beef from KSH because their enteric microflora could acquire resistance genes [42]. Moreover, resistance of the isolates to chloramphenicol, which has been banned for use in animals, is of public health importance [43,44]. This resistance recorded suggests possible exposure of the organisms to chloramphenicol through unlawful use in animals or contamination of the carcasses with resistant organisms from human reservoirs [44,45] or due to cross-resistance acquired from drugs with similar characteristics [44,46,47]. Drug-resistant *Salmonella* have also been isolated from both humans and animals in Nigeria [48,49], poultry carcasses in Senegalese slaughterhouse [50] and beef carcasses in Korean slaughterhouse [51].

Table-1: Occurrence of *Salmonella* in samples obtained from KSH.

Sample type	Number of samples processed	Number of *Salmonella* isolates (%)
Slaughter slab swab	20	17 (25.4)
Dressing table swab	20	10 (14.9)
Washing bucket swab	20	7 (10.4)
Knife swab	20	3 (4.5)
Boot swab	20	10 (14.9)
File swab	20	2 (2.6)
Wheelbarrow swab	20	7 (10.4)
Beef surface swab	20	7 (10.4)
Pieces of beef	20	3 (4.5)
Water	20	2 (2.6)
Total	200	67 (33.5)

Table-2: *Salmonella* load counts of beef and slaughter/processing facilities before and after processing activities in KSH.

Sample type	*Salmonella* count before carcass contact, cfu/cm²	*Salmonella* count after carcass contact, cfu/cm²
Slaughter floor swab	$1.10\times10^{10}\pm1.11\times10^{6a}$	$1.0\times10^{10}\pm1.1x10^{6b}$
Dressing table swab	$1.11\times10^{10}\pm11.1\times10^{6a}$	$1.01\times10^{10}\pm1.1\times10^{6b}$
Washing bucket swab	$1.01\times10^{10}\pm1.01\times10^{6a}$	$0.81\times10^{10}\pm0.10\times10^{6b}$
Knife swab	$1.10\times10^{10}\pm1.10\times10^{6a}$	$1\times10^{10}\pm1.0\times10^{6b}$
Boot swab	$1.10\times10^{10}\pm1.01\times10^{6a}$	$1.01\times10^{10}\pm1.10\times10^{6b}$
File swab	$1.10\times10^{10}\pm1.0x10^{6a}$	$1.0\times10^{10}\pm0.11\times10^{6b}$
Wheelbarrow swab	$1.10x10^{10}\pm1.0\times10^{6a}$	$1.01\times10^{10}\pm0.11\times10^{6b}$

Table-3: Susceptibility of *Salmonella* isolates from beef and slaughter/processing facilities in KSH to different antimicrobials.

Antimicrobial agent	Potency (µg)	Number of isolate, n = 67 (%)		
		Resistant	Intermediate	Susceptible
Ciprofloxacin	5	17 (25.4)	26 (38.8)	24 (35.8)
Ofloxacin	5	18 (27.0)	8 (12.0)	41 (61.0)
Ceftriaxone	30	24 (35.8)	0 (0.0)	43 (64.2)
Ampicillin	10	67 (100.0)	0 (0.0)	0 (0.0)
Amoxicillin/clavulanic acid	20/10	59 (88.1)	0 (0.0)	8 (11.9)
Chloramphenicol	30	40 (59.7)	25 (37.3)	2 (3.0)
Gentamicin	10	23 (34.3)	9 (13.4)	35 (52.3)
Streptomycin	10	31 (46.0)	8 (12.0)	28 (42.0)
Nalidixic acid	30	33 (49.3)	13 (19.4)	21 (31.3)
Nitrofurantoin	300	60 (89.6)	7 (10.4)	0 (0.0)
Trimethoprim/sulfamethoxazole	1.25/23.75	51 (76.0)	8 (12.0)	8 (12.0)

In the present study, isolation of *Salmonella* from the carcasses is an indicator of poor hygiene practices in KSH. The contaminated carcasses from the slaughterhouse could result to infection of in-contact persons [20]. Furthermore, beef originating from the slaughterhouse, if not handled hygienically, could result in the contamination of cooking utensils and ready-to-eat food and in-contact surfaces in homes and food vendor kitchens that source meat from the place. Unhygienic handling of food by vendors which is a major public health concern [38] is common in Nigeria [52]. Handling food without maintaining hygiene has been incriminated as a major source of contamination of food and water in Nigeria [25]. It is possible that contaminated meat contributes significantly to the endemicity of salmonellosis and the increased reports of *Salmonella* induced septicemia in Nigeria [16,53-56]. The observed contamination of the meat with *Salmonella* in KSH could possibly have originated from the processors since slaughterhouse personnel are reported to shed *Salmonella* [45,57]. The contamination may also have originated from the live animals which are known to harbor and shed different bacterial organisms which serve as sources of primary contamination of the carcass at slaughter [58], and *Salmonella* are usually found in the intestines [8]. Isolation of *Salmonella* from beef, as recorded in this study, has also been reported from raw meat samples in Ebonyi State, Nigeria [59], cattle carcass in Algeria [60] and Ethiopia [61], chevon and chicken in India [62], and pig carcasses in France [58]. The isolation of *Salmonella* from meat also points to the degree of contamination of contact surfaces [11]. This is reflected in the values of *Salmonella* counts of the floor and processing equipment that reduced after contact with the carcasses which indicates transfer to the carcasses. The order of decrease in *Salmonella* count after contact with beef, as recorded in this study, could be attributed to their degree of contamination, materials they are made up of, the smoothness of the surfaces, and their temperature during the contact. The concrete slaughter floor and wooden display tables had rough surfaces made so to prevent accidental falls and by cutting knives respectively and this may have resulted in difficulty in proper cleaning and retaining of large quantities of the organism after the daily activities. The WBUs in which the beef were washed also contained large quantities of the organisms that might have been washed off from the heavily contaminated beef. The knife, boot, file, and wheelbarrow retained relatively less quantities of the organism after contact with beef carcasses and this could be attributed to the fact that they are made of metal and plastic whose surfaces are relatively smooth. Moreover, during sharpening which is intermittent among butchers during slaughter in KSH, the friction and subsequent heat generated on the knife and file [63] might have killed some of *Salmonella* organisms on the surfaces. Transfer of *Salmonella* from abattoir equipment to meat during processing has been reported [64]. Furthermore, poor evisceration technique observed in the slaughterhouse is associated with high contamination of the meat, processing equipment, and slaughterhouse environment [45]. It was also observed in the course of this study that waste matter and blood were not properly removed and that there was no subsequent disinfection, and therefore, the slaughter floor may have supported the growth of the organism even after slaughter activities in KSH. In addition, the ability of *Salmonella* to adhere to and form biofilms on surfaces contributes to their survival in the environment [25]. It should be noted that bacterial biofilms form better on rough than smooth surfaces [65]. *Salmonella* was equally isolated from the abattoir floor in Benin, Nigeria [27].

In spite of our findings, the work has some shortcomings: First, the strains of *Salmonella* isolates were not determined. Second, the genes responsible for drug resistance were not identified. These could have helped in understanding the mode of resistance of the isolates to antimicrobials and possibly in tracing the sources of the *Salmonella* contaminations.

Conclusion

This study reports high-level contamination of KSH and meat processed in it with multidrug-resistant *Salmonella*. This is of serious public health importance to the workers in the abattoir that have regular contact with the processed meat as well as the public that source meat from the place. There is a need to educate the workers in the slaughterhouse on good hygiene practices in the processing of meat. The KSH slaughterhouse management should reconstruct it to meet the internationally recommended hygiene standards, provide it with potable water, and ensure its regular cleaning and disinfection.

Authors' Contributions

OUC and SCO collected the samples and conducted the experiments. AJO prepared the manuscript for publication. All the authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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