Original article

Antimicrobial profiles of *Salmonella* species isolated from ruminants slaughtered at the two major abattoirs in Ilorin, Nigeria

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A R T I C L E I N F O

A B S T R A C T

Background: Foodborne illness due to the genus *Salmonella* is one of the major challenges affecting public health worldwide and a threat to socioeconomic activities especially in the developing countries. The present study was carried out to determine the isolation rate and antimicrobial susceptibility profiles of *Salmonella* species from ruminants slaughtered for human consumption at the two major abattoirs in Ilorin, Kwara State. **Methods**: Between November, 2019 and February, 2020, a total 500 samples were collected from the two major abattoirs in Ilorin metropolis (cattle, n = 240; goat, n = 260). *Salmonella* species were isolated and identified using standard bacteriological techniques. The isolates were subjected antimicrobial susceptibility test using Kirby Bauer disk diffusion assay. **Results**: 20 (4 %) *Salmonella* isolates were obtained, there was no significant statistical difference between the isolation rates from cattle (3.75 %) and the small ruminant (4.23 %). Varying degree of resistance patterns were obtained with high proportion of the isolates (> 60 %) displaying resistance to penicillin and cephalosporin tested while low resistant phenotypes (5 %) were observed against quinolones. The isolates were pan-susceptible to gentamicin. **Conclusion**: The study documented multidrug resistant *Salmonella* isolates from ruminants slaughtered for human consumption. It is recommended that further studies to characterize the isolates will assist in recommending the appropriate control strategies to prevent the transmission of resistant *Salmonella* strains to human via food chain.

Introduction

Foodborne illness due to bacteria especially of the genus *Salmonella* is one of the major threats affecting public health worldwide with the developing countries being the most affected because of lack or inadequacies of regular surveillances [1]. Foodborne salmonellosis is reported to be a challenge to socioeconomic activities through hospitalization, treatment and prophylaxis especially among the immunosuppressed individuals (children, elderly, and immunocompromised persons) [1,2]. Annually, gastrointestinal illnesses due to *Salmonella* infection has been reported to be 93.8 million cases worldwide while death is estimated at 155 000 globally [3,4]. Other symptoms associated with

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salmonellosis include nausea, colic, pyrexia and diarrhoea which may subside after some days without medication. However, most cases result in high morbidity or even mortality in affected individual or animals [1,3,5]. In developing countries like Nigeria, data on the burden of foodborne pathogens are either not available or incomplete because of inadequate or lack of regular surveillances [6]. For instance, in Nigeria, the available data on the burden and death associated with human salmonellosis is over a decade ago and it was reported therein that there was increase trend in the cases of human salmonellosis [7]. Death associated with salmonellosis in Lagos State, Nigeria between 1999 and 2008 were up to 880 while 85,187 hospitalizations due to salmonellosis were recorded within the same period [8].

Food-producing animals and their products are major sources of human salmonellosis. Many food animals including cattle [1,9–12], poultry [6,13–15], pigs [5], fish [16] among others have been incriminated as sources of salmonellosis. *Salmonella* may be present in the intestine of wild and domestic animals as commensal or parasite while they serve as reservoirs for human infections [17,18]. Environment plays important roles in the transmission of *Salmonella* to cattle as most of the infections in animals occurs through oro-faecal routes [1,19]. Other sources of infection include fomite, rodents and insects [20,21]. Transportation is another risk factor that may enhance contamination of cattle with *Salmonella* especially when they come in contact with faeces of other carrier animals during transit [1]. Salmonellosis in animal is important because of its direct economic impacts [10]. However, its greater impact is noted in these animal serving as reservoirs for human salmonellosis [13,21]. Human infection occurs through consumption of undercooked faecally contaminated carcass which usually occurs during post slaughtering processes such as evisceration or splitting [1,22]. Many cattle may be presented to abattoirs with asymptomatic *Salmonella* infection and this has been a major threat to public health as it poses serious risk to food chain [1,23]. These sources of *Salmonella* in abattoir can be curtailed by enhancing strict hygiene during slaughtering and post slaughtering processes to reduce contamination of carcasses or meat products and alleviate subsequent burden of salmonellosis.

The health status of an individual largely depends on the wholesomeness of what was consumed. In many developing countries, the safety, hygiene and sanitation of food and food environments are compromised. Food is easily prone to hazards (such as contaminants, adulterants, toxins etc.), leading to foodborne illness, injury and spoilage [24]. These sometimes give rise to conditions that have negative implications on animals and public health. Many of the developing countries do not have meat safety acts [25] or where it exists, there is lack of enforcement of standards of operation [9] while other lack supply of portable water for hygienic abattoir operations [26].

In human, apart from causing foodborne zoonosis, *Salmonella* has also contributed a lot to antimicrobial resistance that is transferred among the organisms [10]. The use and misuse of antimicrobials for treatment, prophylaxis or as feed additive for promoting growth may favour development of multidrug resistant (MDR) bacteria including *Salmonella* which might eventually be transmitted to human through food chain [24]. In Europe and America, it has been documented that death associated with MDR bacterial infections is as high as 2.3×10^4 and 2.5×10^4 respectively [27,28]. Even though, there is no records of estimated death due to MDR bacterial infections in the developing countries including Nigeria, it is believed that the toll will be higher because of indiscriminate and uncontrolled utilization of antimicrobials in veterinary and human practices which may lead to building up of selective pressures and subsequent development of MDR bacteria. In addition, there is insufficient and neglected participation of concern policy makers in the control of environmental resources in the developing countries [28]. Furthermore, multi-drug resistant *Salmonella* has been documented from different food animals slaughtered for human consumption at abattoir [29]. This study aimed at determining the isolation rate and antimicrobial susceptibility profiles of *Salmonella* species from cattle and small ruminants slaughtered for human consumption at the two major abattoirs in Ilorin, Nigeria.

Materials and Methods

Study area and study design
The two abattoirs, Akerebiata abbatoir, where large ruminants are slaughtered and Ipata Abbatoir, where small ruminants are slaughtered (**Figure 1**) were purposefully selected because they were the major abattoirs which serve the people of Ilorin metropolis. Ilorin is an important city in Nigeria, as related to disease epidemiology and transmission,

because it's the transition zone between the Southern and the Northern parts of the country. According to the National Bureau of Statistics, Ilorin has an estimated population of approximately 1,000,000 [30].

Sample collection

A total of 500 samples were collected comprising 240 samples from large ruminant's abattoir and 260 samples from small ruminant's abattoir. The samples spread include faeces (n = 80), kidney (n = 3), liver (n = 148), spleen (n = 101), mesenteric lymph node (n = 30), small and large intestine (n = 138). One sample each was collected per slaughtered animal. Samples were collected aseptically from the slaughtered ruminants as previously described [29].

Approximately, five grams of each sample was collected with sterile scalpel-blade with gloved hands, samples were stored into sterile sample bags and placed in cool box containing ice packs. All samples collected were transported under cold chain and within one hour to Veterinary Microbiology Laboratory, University of Ilorin, for analyses.

Sample processing

Each sample was pre-enriched by inoculating 1 g of the sample in 9 ml of peptone water (Oxoid, Hampshire, UK) and incubated at 37 ± 1 ^{0}C for 24 ± 2 hours. Pre-enriched samples were enriched in in Rappaport-Vassiliadis (RV) (Oxoid, Hampshire, UK) and Selenite-F (SF) broths (Oxoid, Hampshire, UK) at the ratio of 1:9 pre-enriched sample to broths and incubated at 37 ± 1 ^{0}C and 42 ± 1 ^{0}C for 24 ± 2 hours as previously described [15]. Positive results showed a colour change from original blue colour to colourless (RV) or original amber colour to purple (SF) after incubation.

Isolation and biochemical characterization of Salmonella

Salmonella-Shigella Agar (SSA) and Xylose Lysin Deoxycholate Agar (XLD) (Oxoid, Hampshire, UK) were prepared according to manufacturer's directives and enriched samples from RV and SF broths were sub-cultured onto SSA using sterile inoculating wire loop and the plates were incubated at 37 ± 1 ^{0}C for 24 ± 2 hours. Presumptive positive results showed greyish white colonies with dark centres. Presumptive isolates on SSA were further sub-cultured onto XLD agar and incubated at 37 ± 1 ^{0}C for 24 ± 2 hours as previously described [5,13,31]. XLD is a more selective medium for *Salmonella* and positive samples showed pinkish discrete colonies with dark centres. Presumptive isolates on the

selective media (SSA and XLD) were purified by sub-culturing on nutrient agar (Oxoid Ltd, Hampshire, UK) supplemented with 5 % sheep RBC and incubated at 37 ± 1 ^{0}C for 24 ± 2 hours. Pure isolates were kept in Mueller Hinton broth containing 20 % glycerol at -70 ^{0}C until needed.

Standard biochemical tests for presumptive identification of *Salmonella*, including triple sugar iron (TSI) (to detect H_2S, gas production, and sugar fermentation), urease, indole, methyl red, Voges–Proskauer, citrate, catalase and motility tests as previously described [32].

Phenotypic characterization of antimicrobial profiles of the isolates

Phenotypic antimicrobial profiles of the isolates were determined using Kirby--Bauer disk diffusion assay on Mueller Hinton agar (Oxoid, Hampshire, UK) as previously described [33] The panel of antibiotics discs (Oxoid, Hampshire, UK) used in the assay have the following antibiotics and concentrations; ciprofloxacin (5 µg), nalidixic acid (30 µg), gentamicin (10 µg), tetracycline (30 µg), compound sulphonamides (300 µg), ampicillin (10 µg), oxacillin (10 µg), ceftazidime (30 µg), ceftriaxone (30 µg) and sulfamethoxazole trimethoprim (25 µg).

Briefly, stock isolates were sub-cultured onto freshly prepared nutrient agar (Oxoid, Hampshire, UK) from which one to two discrete colonies were inoculated into 5ml of sterile distilled water in test tubes using sterile wire loop. It was allowed to stand for approximately 5 minutes after which the turbidity was adjusted to 0.5 McFarland standard. The inoculum was uniformly poured on the Mueller Hinton agar plate. The plates were partially left open for 3-5 minutes to dry. Antibiotics sensitivity disks were then dispensed on each plate using a disc dispenser (Oxoid, Hampshire, UK). The plates were subsequently incubated at 37 ^{0}C for 16 - 24 hours. The zones of inhibition of each antibiotic was measured and recorded according to clinical and laboratory standard institute (CLSI) standard. The zones of inhibition were interpreted as sensitive, intermediate or resistance according CLSI guidelines [34]. *Escherichia coli* CCM 3954 was used for techniques and protocol validations.

Results

The studies revealed that out of the 500 samples collected, 20 (4 %) were positive for *Salmonella*. The rate of isolation varied between the species sampled with 9 (3.75%) being positive among the cattle while 11 (4.23 %) were positive

among the goats, even though, the variation was not statistically significant (*p*>0.05). Similarly, no statistically significant difference between isolation rate in males 2 (0.83 %) and female animals 7 (2.91 %) albeit, higher rate was obtained from females **(Table 1)**. Based on sample type, *Salmonella* was obtained from mesenteric lymph node, intestine, and faeces at the rate of 6.7 % (n=2), 5.1 % (n=7) and 5 % (n=5) respectively **(Table 2)**. Bio-typing revealed all the isolates were Gram negative rods, catalase positive, oxidase and indole negative and produce hydrogen sulphide from TSI **(Table 3)**. Varying degree of resistance patterns were obtained; 95 % (n = 19) of the isolates exhibiting resistance to oxacillin, 80 % (n = 16) displayed resistance to each of ceftriaxone and ampicillin while 65 % (n = 13) exhibited resistance to ceftazidime. However, there is reduced resistance (5 % each) to fluoroquinolones

(ciprofloxacin and nalidixic acid) tested in this study while isolates were pan-susceptible to gentamicin **(Table 4)**. A total of ten resistance phenotypes were obtained with amp/caz/cro/ox being the most common (20 %). 25 % (n = 5) of the isolates showed resistance to two sets of antimicrobials and these isolates were obtained from faeces, intestine and lymph nodes. Six (30 %) isolates obtained from faeces, intestine, liver and spleen displayed resistance to three antimicrobials. One isolate (5 %) obtained from spleen exhibited resistance to seven out of the ten tested antimicrobials while 25 % of the isolates displayed multidrug resistance phenotypes. The MDR *Salmonella* isolates were obtained from all the sample sources except lymph node **(Table 4)**.

Figure 1. Map of Kwara State showing the abattoirs locations in Ilorin, Kwara State, Nigeria.
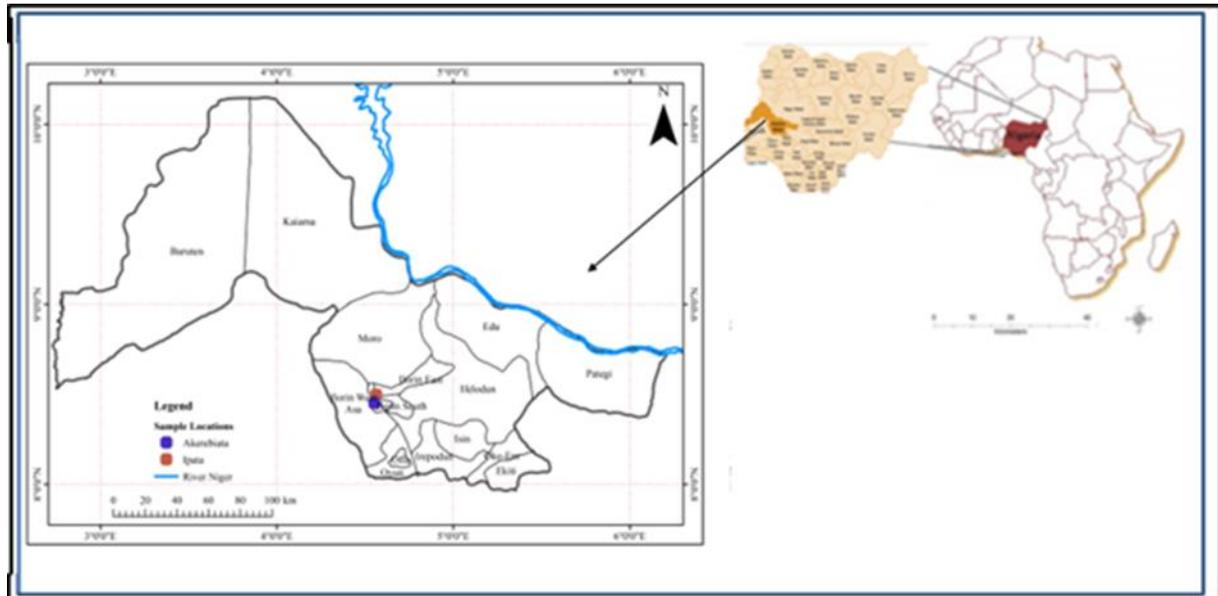

Table 1. Isolation rate of *Salmonella* species from ruminants slaughtered at the two major abattoirs in Ilorin, Kwara State.

S/N	Species	Sex	Number of Sample	No of positive Sample (%)	*p*-value
1	Cattle	Male	51	2 (0.83)	0.9421
		Female	189	7 (2.91)	
	Sub Total		**240**	**9 (3.75)**	
2	Goat	Male	260	11 (4.23)	NA
		Female	0	0 (0)	
	Sub Total		**260**	**11 (4.23)**	
	Total		**500**	**20 (4)**	0.7840

Table 2. Organ distribution of *Salmonella* species isolated from ruminant slaughtered at the two major abattoirs in Ilorin, Kwara State.

S/N	Sample type	No of Samples	No of positive (%)
1	Faeces	80	4 (5.0)
2	Intestine	138	7 (5.1)
3	Kidney	3	0 (0.0)
4	Liver	148	3 (2.0)
5	Mesenteric lymph node	30	2 (6.7)
6	Spleen	101	4 (4.0)
Total		500	20 (4.0)

Table 3. Biochemical characteristics of *Campylobacter* isolates from ruminants slaughtered at major abattoirs in Ilorin, Kwara State, Nigeria.

Source of sample	No of positive	Biochemical characteristics of the isolates											Detection (%)
		GRA	URE	OXI	CAT	GLU	LAC	H_2S	Indole	MR	Cit	Mot	
Akerebiata	9	G-ve$^-$	-	-	+	+	-	+	-	+	+	+	0.09
Ipata	11	G-ve$^-$	-	-	+	+	-	+	-	+	+	+	0.11
Total	20												0.20

Key: GRA= Gram reactions, G-ve$^-$=Gram negative rods, URE=urease, OXD=oxidase, CAT=catalase, GLU=glucose, LAC=lactose, -=negative, +=positive H_2S= hydrogen sulphides, MR= Methyl red, Cit= Citrate, Mot= Motility.

Table 4. Antimicrobial susceptibility profiles of *Salmonella* isolates from ruminants slaughtered at the two major abattoirs in Ilorin, Kwara State.

Phenotypes	No	Sources (n)	Class of antimicrobials resisted (n)	MDR status
amp/ox	3	Lymph Node (1) Faeces (1), Intestine (1)	Penicillin (1)	Absent
caz/cro	1	Lymph Node (1)	Cephalosporin (1)	Absent
caz/ox	1	Intestine (1)	Cephalosporin-Penicillin (2)	Absent
amp/cro/ox	3	Faecal (1), Intestine (1), Spleen (1)	Cephalosporin-Penicillin (2)	Absent
caz/cro/ox	3	Faecal (1), Liver (1), Intestine (1),	Cephalosporin-Penicillin (2)	Absent
amp/caz/cro/ox	4	Liver (1), Intestine (2), Spleen (1),	Cephalosporin-Penicillin (2)	Absent
amp/cro/ox/te	1	Intestine (1)	Cephalosporin-Penicillin-Tetracycline (3)	Present
amp/caz/cro/ox/te	1	Liver	Cephalosporin-Penicillin-Tetracycline (3)	Present

Table 4. Continued.

Phenotypes	No	Sources (n)	Class of antimicrobials resisted (n)	MDR status
amp/caz/cip/cro/ox/sxt	1	Faecal	Cephalosporin-Fluoroquinolone-Penicillin-Sulphonamide (4)	Present
amp/caz/cro/na/ox/te	1	Spleen	Cephalosporin-Penicillin-Quinolone- Tetracycline (4)	Present
amp/caz/cro/ox/te/sxt/s₃	1	Spleen	Cephalosporin-Penicillin- Sulphonamide- Tetracycline (4)	Present

Key: cip= ciprofloxacin, na= nalidixic acid, gentamicin, te= tetracycline, s_3= compound sulphonamides, amp= ampicillin, ox= oxacillin, caz= ceftazidime, cro= ceftriaxone, and sxt= sulfamethoxazole trimethoprim

Discussion

In the current study, *Salmonella* was isolated from all the sample types at varying rates. The overall rate of *Salmonella* isolation from slaughtered ruminants is lower than the rate reported in Maiduguri (39.0 %), north eastern Nigeria by **Jajere et al.** [29]. The reason may be related to Maiduguri being a border to many countries, moreover, most of the ruminants that are slaughtered at Maiduguri abattoirs may have been sourced from different neighbouring countries as reported previously [35]. Similarly, the rate of *Salmonella* reported from slaughtered cattle alone is lower and agree with 3.0 to 7 % previously reported [1,9–12]. The presence of *Salmonella* in the slaughtered animals may be attributed to faecal contaminations or contact of carcass product with contaminated surfaces such as abattoir floors, hides and skin, or butchers' equipment [9]. The differences in isolated rates reported in different regions may be due to difference in geographical locations, season of study, study protocol and the level of hygiene practiced at the abattoirs. The presence of *Salmonella* in food animals and food of animal origin pose serious risk to public health [13]. In addition, *Salmonella* has been reported to cause serious economic loss to farmers through morbidity and mortality of affected animals [1,13].

High rate of isolation of *Salmonella* from intestinal contents is in agreement with previous study [1] and could be attributed to some cattle that may be asymptomatic carriers which are continually shedding the organism [1,36]. These carrier animals may, in addition to serving as sources of transmission of *Salmonella* infections in the herd, serve as sources of human foodborne salmonellosis [1]. The isolation rate of *Salmonella* from intestinal content, mesenteric lymph nodes, liver and spleen corroborated previous study [10] and contamination of the liver, spleen and the intestine could serve as source of human infections through food chain via the consumption of raw or undercooked visceral as delicacies. The presence of *Salmonella* in the lymph node indicated the systemic nature of the infections in some of the slaughtered animals and this could be of importance in animal and veterinary practices because many antimicrobials do not reach lymph nodes during distribution, hence, resultant therapeutic failure when administered.

In addition, the rate of isolation from male animals is not statistically different ($p>0.05$) from that of females. This corroborate with report of previous study which revealed that salmonellosis has no gender specificity, especially in immunocompetent old animals [37].

The isolates in this study generally showed high resistance to penicillin and cephalosporin and the finding is in agreement with previous studies in Nigeria where MDR *Salmonella* isolates from cattle slaughtered in abattoirs were reported to have exhibited resistance to routinely prescribed antibiotics for treatment of human salmonellosis such as oxacillin/clavulanic acid, chloramphenicol, ampicillin, tetracycline, penicillin, and erythromycin [9,10,12]. This phenomenon could be due to selective pressure as a result of use and misuse of antibiotics in veterinary and animal productions for prophylaxis, therapeutics or as growth promoters. The high resistance to the drugs such as penicillin and cephalosporin groups is a cause of public health concern as these are among the major drugs usually prescribed for the treatment of salmonellosis in human. The low resistant profiles of these isolates to the quinolones tested in the current study could be due to the low level of prescription or administration of these antimicrobials ruminant production in Nigeria [38]. Because over 50 % of the isolates showed resistance to two or more antimicrobials and 25 % of the isolates displayed MDR phenotypes, this is a serious public health concern as the infected food animals are the major sources of foodborne disease transmission to human.

In conclusion, this study reported a low-level of *Salmonella* contamination rate in ruminants slaughtered at the two major abattoirs in Ilorin, this may be due to improved hygienic practices observed among the abattoirs' workers such as washing of the carcass with portable water, rinsing of butchers' working implements such as knives, at interval among others. The isolates showed high resistance to β-lactam antibiotics, which may be due to the abuse of these agents in veterinary practice and animal production. Hence, there is need for consumers' enlightenment on the impart of antimicrobial resistance and the need for proper cooking of foods of animal origin before consumption. Further detailed studies, involving molecular characterization of *Salmonella* isolates from different abattoirs are recommended, this could generate a more robust data such as serovars distribution, resistance genes types, multi locus sequence typing (MLST) and plasmids types which could be utilized for development of strategies to

prevent transmission of *Salmonella* to human through food chain.

Authorship

A.O.A., R.A.I and R.A.M. were responsible for the conception and design of the study. P.T.B., A.O.A., I.A.R. and M.A.S. were responsible for acquisition, analyses and interpretation of data. Drafting of the article and revising it critically for important intellectual content was carried out by A.O.A., A.A., P.T.B, S.A. and A.G.J. while final approval of the version to be submitted was done by R.A.I., A.O.A. and A.U.

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