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Occurrence and antimicrobial susceptibility patterns of *Salmonella* species from poultry farms in Ibadan, Nigeria

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Background: *Salmonella* species are among the major foodborne pathogens causing diseases of economic and public health implications in poultry and humans globally.

Objective: This study aimed to determine the occurrence and antimicrobial susceptibility patterns of *Salmonella* isolates from chickens in poultry farms in Ibadan, Southwest Nigeria.

Methods: Cloacal swab samples (n = 360) were obtained from chickens randomly selected from 10 poultry farms in five local government areas of Ibadan, Oyo State, from 04 April 2018 to 20 November 2018. Bacterial identification and antimicrobial susceptibility testing were performed using established protocols. Data were analysed using descriptive statistics and Pearson's chi-squared test at $P \leq 0.05$ significance level.

Results: The overall prevalence of *Salmonella* was 21.4%. There were statistically significant associations between *Salmonella* prevalence and the farm location ($p = 0.003$), age of chickens ($p < 0.001$), and health status of chickens ($p < 0.001$). All *Salmonella* isolates (n = 77; 100.0%) were resistant to cefuroxime. The isolates were also highly resistant to cotrimoxazole (n = 74; 96.1%), chloramphenicol (n = 73; 94.8%), meropenem (n = 72; 93.5%), gentamicin (n = 69; 89.6%), and tetracycline (n = 64; 83.1%).

Conclusion: The presence of drug-resistant *Salmonella* in commercial layer chickens in Ibadan is a potential threat to consumer health as it increases the risk of carcass contamination and pathogen propagation, and limits the options to control and treat infections in humans and animals. Well-integrated national surveillance systems for monitoring *Salmonella* and antimicrobial resistance in poultry are critical.

Keywords: *Salmonella* species; antibiotics; antimicrobials; resistance; poultry and infection.

Introduction

Salmonella species is one of the primary causes of foodborne illnesses in humans globally.[1] Although most cases of salmonellosis are mild, some may lead to life-threatening enteric fever or an invasive form of the disease requiring antimicrobial treatment, especially among immunocompromised people, infants, and the elderly.[2,3] The consumption of food animal products such as poultry meat has been implicated in human salmonellosis outbreaks worldwide.[4]

The *Salmonella* genus comprises two species, *Salmonella bongori* and *Salmonella enterica*, and over 2500 different serotypes or serovars.[5] In Africa, non-typhoidal salmonellae are common causes of bacterial bloodstream infections in humans with clinical signs of fever[6] and are associated with case fatality rates of about 20.0% – 25.0%.[7] A report from Nigeria showed that non-typhoidal salmonellae (43.5%) and typhoidal salmonellae (56.5%) were isolated from blood and stool samples collected from adults and children.[8]

Poultry farming is a major source of livelihood for poor rural households and urban areas in many developing countries, including Nigeria.[8,9] The poultry sector has the potential to promote national economic growth and provide employment opportunities for Nigerians.[10] For instance, poultry farming serves as an additional occupation to supplement the income of small farm families.[10] Similarly, the poultry value food chain accounts for about a quarter of local meat production in Nigeria and promotes the per capita animal protein consumption of the population.[11] Despite these advantages, small-scale poultry farmers in Nigeria lose up to 18% of chicks in the first two weeks of rearing, often due to salmonellosis; this negatively impacts food security and the socio-economic status of farmers.[12]

The expansion of the poultry industry has increased the occurrence of salmonellosis, which has become an important public health threat in Nigeria, causing heavy economic loss and higher human and animal morbidities and mortalities.[13,14] The most prevalent human *Salmonella* serovars are also common in poultry, suggesting a possible epidemiologic interface between human infections and poultry as a reservoir of *Salmonella*.[8] *Salmonella* can contaminate poultry and propagate among breeding flocks and at all stages of the production cycle, including during hatching, transportation, slaughtering, processing and retailing, and subsequently spread to humans.[15]

Antimicrobial resistance is an emerging global threat associated with the reduced efficacy of antimicrobials in the treatment of human and animal infections, prolonged morbidity, and death.[16] Multidrug-resistant organisms are an increasing challenge to human and livestock health worldwide, and Nigeria is not excluded from the antimicrobial resistance burden. Antimicrobials are used in veterinary practice as growth promoters, prophylactics or therapeutics. The indiscriminate use of antimicrobials has been linked to increased antimicrobial resistance among bacterial pathogens isolated from poultry and the poultry environment, including *Salmonella*.[17] Therefore, poultry may harbour multidrug-resistant pathogens, which could be transmitted to human populations anywhere through direct contact or by consumption. Hence, our study focused on determining the occurrence and antimicrobial susceptibility patterns of *Salmonella* isolates from chickens in poultry farms in Ibadan, Oyo State, Nigeria.

Methods

Ethical considerations

The Animal Care and Use Research Ethics Committee (ACUREC), University of Ibadan, Nigeria, reviewed and approved the protocols of the study (UI-ACUREC/18/0105). Verbal informed consent was obtained from farm owners before sampling chickens.

Study design and sampling method

A cross-sectional study was conducted to determine the occurrence of multidrug-resistant *Salmonella* in poultry farms in Ibadan, Oyo State, Southwest Nigeria. A multistage sampling technique was used to select the chickens for sampling. First, five local government areas (LGAs) in Ibadan metropolis, namely Ibadan North, Ibadan North East, Ibadan North West, Ibadan South West and Ibadan South East were randomly selected for the study. Next, a total of 10 commercial layer poultry farms (A–J), two from each of the five LGAs, were purposively recruited based on the presence of commercial layer production and farmers' consent. Afterwards, a single pen from each farm was randomly selected, from which 36 chickens were sampled by systematic random sampling. The population of the poultry farms ranged from 500 to 2000 chickens aged between 15 and 36 weeks at the time of sampling; the breeds raised were Isa Brown and Bovans Nera Black. The farms all operated an intensive management system using battery cages.

Sample size

Using a prevalence of 3.5% of *Salmonella* species reported in layers from poultry farms in Ilorin, Kwara State,[18] the sample size was calculated using the formula,[19]

$$n = z^2p(1\text{-}p)/d^2 \quad \text{[Eqn 1]}$$

where n = minimum sample number, z = confidence interval at 95% (1.96), p = prevalence level of subject (3.5%), and d = level of precision (2%). A sample size of 324 was determined, and a 10% non-contingency was added to make up for non-response, giving a minimum target sample size of 360.

Sample collection

All sanitary and biosecurity protocols were observed by investigators to avoid the spread of pathogens between farms during sample collection. Farm visits were conducted at about 2-week intervals over eight months from 4 April 2018 to 20 November 2018. Each farm was visited once by the investigators and 36 samples were collected during each visit. The investigators visited each farm on dates specified by the farm managers to ensure that no biosecurity measures were compromised. A systematic random sampling was performed to collect samples from chickens within the pens irrespective of age, breed, and health status. A total of 360 cloacal swabs were collected from live chickens (layers) for the detection of *Salmonella*. Samples from farms A and C were taken from diseased chickens as the farmers refused access to the apparently healthy ones.

Cloacal swab samples were collected by gently rotating the cotton swab within the cloacae, with care taken to avoid contact with other areas to prevent contamination. All samples were transported in sterile 10 mL tubes on ice at 4 °C to the Veterinary Public Health Laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, for bacteriological analysis. No transport medium was used. Samples were processed immediately on arrival at the laboratory. For each sampled chicken, information on the species, breed, age, health status, management system, and recent antibiotics administered was also obtained and documented.

Isolation of *Salmonella*

Isolation of *Salmonella* was carried out according to the International Organization for Standardization standard 6579 guidelines[20] for isolation and characterisation of *Salmonella* species. Briefly, pre-enrichment was conducted by transferring each swab into 10 mL tubes containing buffered peptone water (Oxoid, Basingstoke, United Kingdom). Each tube was then thoroughly homogenised by vortexing and incubated at 37 °C for 18–20 h. Subsequently, 0.1 mL of the pre-enrichment broth culture was inoculated into 10 mL of

Rappaport Vassiliadis broth (Oxoid, Basingstoke, United Kingdom) for the selective enrichment of *Salmonella* and incubated at 42 °C for 24 h. A loopful from the overnight enrichment broth was then inoculated onto xylose lysine deoxycholate agar (Oxoid, Basingstoke, United Kingdom) and brilliant green agar (Oxoid, Basingstoke, United Kingdom), and the plates were incubated at 37 °C for 24 h. To obtain pure colonies, the presumptive *Salmonella* colonies that appeared red with black centres on xylose lysine deoxycholate agar and pinkish-white or red surrounded by a red halo on brilliant green agar were subcultured on xylose lysine deoxycholate agar plates and incubated at 37 °C for 24 h. The pure cultures were subsequently inoculated on nutrient agar slants, incubated at 37 °C for 24 h and stored in the fridge at 4 °C.

Phenotypic characterisation of *Salmonella*

Salmonella isolates were identified based on colony morphology, and further phenotypically confirmed and characterised using Gram staining (negative) and standard laboratory biochemical tests such as indole (negative), methyl red (positive), Voges-Proskeur (negative), citrate (negative), oxidase (negative), catalase (positive), urease (negative), and triple sugar iron agar (acid/alkaline). Also, sugar fermentation tests for glucose (positive), lactose (negative), mannitol (positive), sucrose (negative), and xylose (positive) were performed.

Antimicrobial susceptibility tests

The antimicrobial susceptibility test was carried out using the agar disk diffusion method.[21] The sensitivity patterns of the cultured isolates were reported for standard antibiotics according to the Clinical Laboratory Standards Institute.[22] Briefly, putative *Salmonella* isolates on nutrient agar slants were subcultured onto nutrient agar plates and incubated for 24 h at 37 °C. Subsequently, a colony from each plate was emulsified into sterile saline to achieve turbidity equivalent to 0.5 McFarland standard (~ 10^8 CFU/mL). The inoculum was aseptically and uniformly spread on the surface of prepared Muller Hinton agar plates (Oxoid, Basingstoke, United Kingdom). Thereafter, the antibiotics disks (Biomark Laboratories, Pune, Maharashtra, India) were placed aseptically on the inoculated agar surface using sterile forceps and the plates were incubated at 37 °C for 16–18 h. The following antimicrobials were used: tetracycline (10 µg), cotrimoxazole (25 µg), gentamicin (10 µg), cefuroxime (30 µg), chloramphenicol (10 µg), ciprofloxacin (5 µg), meropenem (10 µg) and amikacin (30 µg). The zones of inhibition around each disk were measured and interpreted according to the Clinical Laboratory Standards Institute guidelines. The *Escherichia coli* American Type Culture Collection 25922 reference strain was used as the control. An isolate was considered multidrug-resistant if resistant to at least one antimicrobial in three or more antimicrobial classes.

Data analysis

Data were analysed using descriptive statistics such as proportions and percentages and presented as a table and figures. A Pearson's chi-squared (χ^2) test and odds ratios were computed to test the association between the presence of *Salmonella* and age, breed, farm location, and health status of the chickens. The level of significance was set at $p \leq 0.05$. All statistical analyses were conducted using the Statistical Package for the Social Sciences version 21 (IBM Corp., Armonk, New York, United States, 2012).

Results

A total of 77 (21.4%) of the 360 samples were positive for *Salmonella*. Farm C, one of the farms in Ibadan North-West where only sick chickens were sampled, had the highest *Salmonella* prevalence (21/36; 58.3%) (Figure 1).

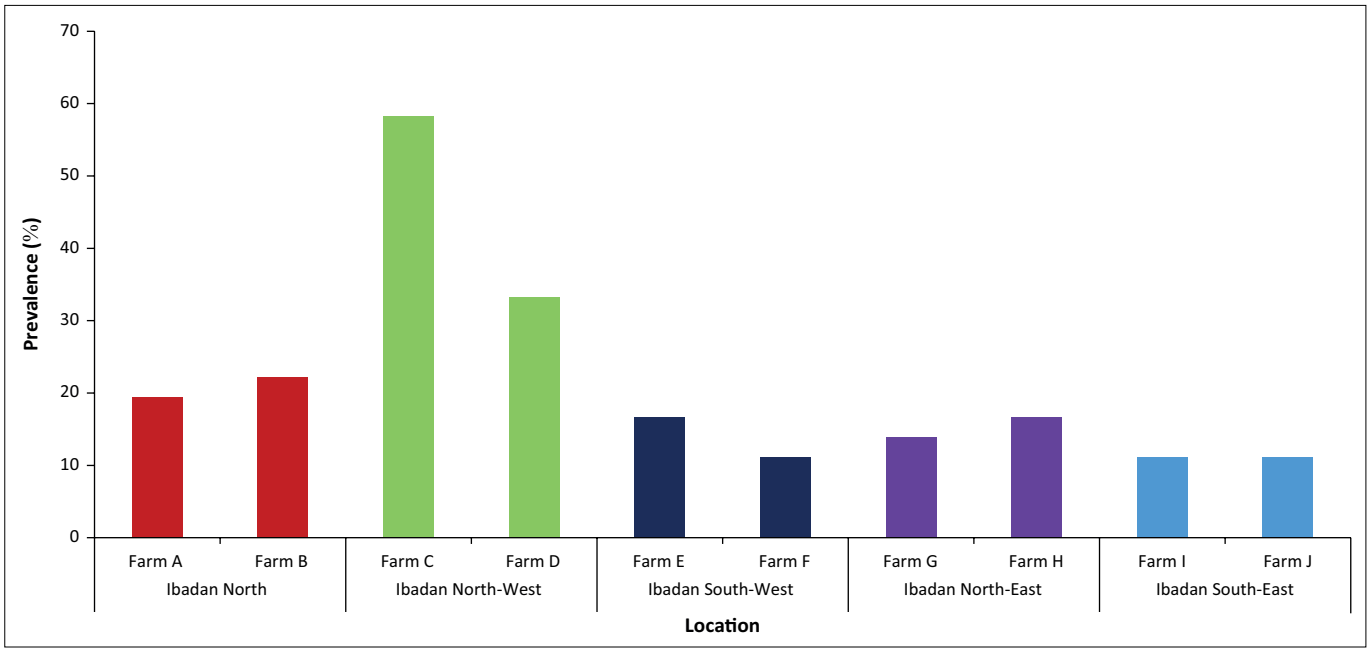

FIGURE 1: Occurrence of *Salmonella* species in chickens from poultry farms in Ibadan metropolis, Nigeria, April 2018 – November 2018.

TABLE 1: Association between *Salmonella* prevalence and demographic variables of chickens from poultry farms in Ibadan metropolis, Nigeria, April 2018 – November 2018.

Characteristic	Variables	*Salmonella* detected		*Salmonella* not detected		OR	95% CI	*p*
		N	%	*N*	%			
Breed	Isa Brown (*n* = 216)	51	23.6	165	76.4	1.40	0.83–2.38	0.26
	Bovans Nera Black† (*n* = 144)	26	18.1	118	81.9	Ref	-	-
	Total (*n* = 360)	77	-	283	-	-	-	-
Age distribution	15–20 weeks† (*n* = 72)	8	11.1	64	88.9	Ref.	-	-
	21–26 weeks (*n* = 108)	39	36.1	69	63.9	4.52	1.97–10.40	< 0.001*
	27–32 weeks (*n* = 72)	11	15.3	61	84.7	1.44	0.54–3.83	0.62
	33–38 weeks (*n* = 108)	19	17.6	89	82.4	1.71	0.70–4.14	0.33
	Total (*n* = 360)	77	-	283	-	-		-
Health status	Sick (*n* = 72)	28	38.9	44	61.1	0.32	0.18–0.57	< 0.001*
	Healthy† (*n* = 288)	49	17.0	239	83.0	Ref.	-	-
	Total (*n* = 360)	77	-	283	-	-	-	-
Location	Ibadan North† (*n* = 72)	15	20.8	57	79.2	Ref.	-	-
	Ibadan North-West (*n* = 72)	33	45.8	39	54.2	3.22	1.54–6.70	0.003*
	Ibadan South-West (*n* = 72)	10	13.9	62	86.1	0.61	0.26–1.47	0.38
	Ibadan North-East (*n* = 72)	11	15.3	61	84.7	0.69	0.29–1.62	0.52
	Ibadan South-East (*n* = 72)	8	11.1	64	88.9	0.48	0.19–1.20	0.17
	Total (*n* = 360)	77	-	283	-	-	-	-

OR, odds ratio; CI, confidence interval.
*, *P*-values ≤ 0.05 are statistically significant.
†, Reference group.

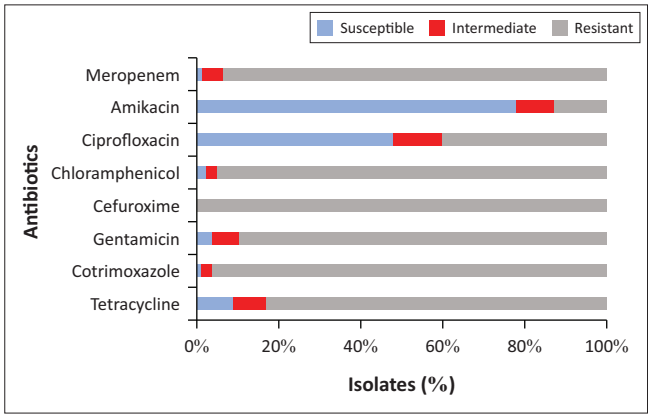

FIGURE 2: Antimicrobial resistance profile of *Salmonella* isolates from chickens from poultry farms in Ibadan metropolis, Nigeria, April 2018 – November 2018.

Overall, the prevalence of *Salmonella* was higher among chickens of the Isa Brown breed (51/216; 23.6%) and chickens aged between 21 and 26 weeks (39/108; 36.1%) (Table 1).

Age, farm location, and health status of chickens were all associated with *Salmonella* carriage. Layer chickens aged between 21 and 26 weeks had four times the odds of *Salmonella* carriage compared to those aged between 15 and 20 weeks (odds ratio = 4.52; 95% confidence interval: 1.97–10.4, $p < 0.001$). Similarly, chickens in farms in Ibadan North-West had three times higher odds of being *Salmonella*-positive compared to those from Ibadan North LGA (odds ratio = 3.22; 95% confidence interval: 1.54–6.7; $p = 0.003$). Farms in Ibadan North-West LGA operated under poor hygienic and low biosecurity conditions. Interestingly, sick chickens had lower odds of harbouring *Salmonella* than the healthy ones (odds ratio = 0.32; 95% confidence interval: 0.18–0.57; $p < 0.001$).

Antimicrobial resistance profiles of the *Salmonella* isolates

All of the isolates ($n = 77$; 100.0%) were resistant to cefuroxime. Isolates were also highly resistant to cotrimoxazole ($n = 74$; 96.1%), chloramphenicol ($n = 73$; 94.8%), meropenem ($n = 72$; 93.5%), gentamicin ($n = 69$; 89.6%) and tetracycline ($n = 64$; 83.1%), but only moderately resistant to ciprofloxacin ($n = 31$; 40.3%) (Figure 2).

Discussion

Food safety issues, including the presence of zoonotic and antibiotic-resistant pathogens in farm animals, are of increasing concern among consumers of animal food products globally. We reported a 21.4% prevalence of *Salmonella* in commercial poultry in Ibadan. Previous studies have reported the prevalence of *Salmonella* infection in poultry farms and chickens in Nigeria to be between 2.0% and 43.0%,[17,23,24,25,26,27,28,29,30,31,32] and this is similar to the prevalence reported in another study conducted in Bangladesh.[33] *Salmonella* have been implicated in bacteraemia, septicaemia and gastroenteritis in humans, and their presence in poultry chickens may pose serious health risks to humans.[34] The observed *Salmonella* prevalence in the farms studied may be due to poor biosecurity measures, contamination from poultry premises or feed, or bacterial transmission via faecal matter by insects and rodents.[35] Chickens from poultry farms in Ibadan North-West LGA had three times the odds of being colonised by *Salmonella* and this may be attributed to the unhygienic and poor sanitary measures observed on the farms during site visits, including the presence of heaps of faecal matter in the pens and absence of disinfectant foot dips. *Salmonella* transmission in farms may also be promoted by inadequate knowledge and poor attitudes towards biosecurity and poultry management.[36] Similarly,

as observed, leaving dead carcasses in the farm, lack of proper hygiene, and the presence of faeces in water could have accounted for the high prevalence of *Salmonella* in farms in Ibadan North-West. Similar to past studies conducted in Nigeria, Colombia and Baghdad,[37,38,39] there was no association between chicken breed and *Salmonella* colonisation in our study.

The observed high rates of resistance to antimicrobials among the isolates is not surprising as most poultry farmers in the country have unlimited over-the-counter access to antimicrobials and indiscriminately use them as growth promoters and prophylactics[40] without veterinary prescriptions.[41,42] The ciprofloxacin resistance was lower in this study compared to another study conducted in Nigeria,[43] but similar to a report from the United States.[44]

All *Salmonella* isolates were resistant to cefuroxime, which was the only cephalosporin tested in this study; this is similar to previous reports from Nigeria, the United Arab Emirates, Germany and India.[17,45,46] This poses a serious threat to humans as cephalosporin-resistant *Salmonella* can potentially be transmitted to humans via the consumption of such poultry or poultry products. Moreover, there are limited treatment options for human infections caused by pathogens resistant to multiple classes of antimicrobials, particularly those involving the cephalosporins and fluoroquinolones.[46]

The high resistance to tetracycline documented in this study is not surprising. Tetracycline is the most abused antibiotic for the treatment and prevention of poultry disease in Nigeria.[17,40,41] Nevertheless, the tetracycline resistance rate in this study is higher than those reported in past studies in Ghana[47] and Cote d'Ivoire,[48] possibly because of differences in geographical location, farm biosecurity levels, and national implementation of antimicrobial policies in these countries.

Limitations

We were not able to identify the specific serovars of the *Salmonella* isolates due to non-availability of antisera to perform serotyping. Also, the findings of this study are not generalisable to farms in Ibadan as only the five LGAs in Ibadan metropolis were sampled. Further work, which should involve the detailed sampling of more LGAs and serotyping of *Salmonella* isolates, is needed to provide more insight into the prevalence of the various serovars circulating in the poultry population in Ibadan, Nigeria.

Conclusion

We report a high prevalence of *Salmonella* among chickens from poultry farms in Ibadan, Nigeria. Apparently healthy and sick chickens may serve as potential shedders and propagators of drug-resistant *Salmonella*, with possible implications for consumer health and the environment. Data generated from this study could inform policies or guidelines for the improvement of on-farm hygiene and biosecurity best practices among poultry farmers to reduce the burden of *Salmonella* in the industry. Also, Nigeria urgently needs well-coordinated or integrated national surveillance systems and networks to monitor antimicrobial use and resistance in animal and human populations and the environment.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

O.O.I. conceived and designed the study. T.G.O., O.O.I. and O.O.A. undertook the methodology of the study. T.G.O. collected, analysed the samples and did the statistical analysis under the supervision of O.O.I. and O.O.A. Furthermore, T.G.O. drafted the original manuscript, and O.O.I. and O.O.A. revised it. All of the authors approved the final manuscript.

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Data availability

The data that support the findings of this study can be made available by the corresponding author, O.O.I., upon reasonable request.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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