Iranian Journal of Veterinary Science and Technology

Received: 2024- Nov-22
Accepted after revision: 2025- Jul-30
Published online: 2025- Jul-30

RESEARCH ARTICLE

DOI: 10.22067/ijvst.2025.90724.1440

Isolation Rate and Antimicrobial Profiles of *Salmonella* from Captive Wild Animals at University of Ilorin Zoological Garden, Kwara State, Nigeria

Ahmed O. Akeem[a], Katibi A. Abdulhameed[a], Sagaya A. Ahmad[a], Adamu L. Fateema[b], Sa'ad B. Abdulkareem[c], Aremu Abdulfatai[d], Abubakar M. Imam[e], Raufu A. Ibrahim[a]

[a] Department of Veterinary Microbiology, University of Ilorin, Ilorin, Nigeria.
[b] Department of Veterinary Microbiology, University of Maiduguri, Ilorin, Nigeria.
[c] Department of Microbiology, University of Ilorin, Ilorin, Nigeria.
[d] Department of Veterinary Pharmacology and Toxicology, University of Ilorin, Ilorin, Nigeria.
[e] Department of Theriogenology and Production, University of Ilorin, Ilorin, Nigeria.

ABSTRACT

Salmonellosis, a globally distributed zoonotic disease, has poorly understood epidemiology in captive wildlife, particularly in developing countries. This study aimed to determine the isolation rate and antimicrobial susceptibility profiles of Salmonella species from captive wildlife at the University of Ilorin Zoological Garden, Kwara State, Nigeria. A cross-sectional study was conducted using 191 faecal samples collected from different species of captive wild animals. Samples were processed using standard bacteriological procedures and antimicrobial sensitivity testing was conducted using the Kirby-Bauer disk method. Out of the 191 samples analyzed, 19 (10.0 %) were positive for Salmonella. The frequencies of isolation varied among different animal classes, with the avian species showing the highest rate (5.24 %). Differences in isolation rates within and between different animal classes were not statistically significant ($p > 0.05$). The isolates generally showed a low level of antimicrobial resistance except against ampicillin (94.7%) and erythromycin (89.5%). Statistically significant resistance was observed for erythromycin ($p = 0.042$) and tetracycline ($p = 0.035$) among all the isolates. Similarly, resistance to ceftazidime was prominent among the primate species sampled ($p = 0.002$). We identified nine distinct resistance profiles, with 15.8% of resistant isolates exhibiting multidrug-resistant (MDR) phenotypes. Notably, 94.7% of Salmonella isolates demonstrated a multi-antibiotic resistance (MAR) index ≥ 0.2. These findings confirm the presence of Salmonella in captive wildlife at this facility, indicating a potential public health risk. Despite generally low antimicrobial resistance levels, ongoing surveillance is crucial to identify infection sources and prevent environmental contamination by MDR zoonotic pathogens.

Keywords

Captive wildlife, Salmonella, MDR, Zoo, Ilorin, MAR Index

Number of Figures: 1
Number of Tables: 2
Number of References:: 17
Number of Pages: 8

Abbreviations

MAR: multi-antibiotic resistance
MDR: multidrug-resistance
ONPG: ortho-nitrophenyl galactosidase
CLSI: Clinical and Laboratory Standard Institute

Corresponding author: *Akeem O. Ahmed*
Email: *ahmed.oa@unilorin.edu.ng*
Tel: *+23(470)6850-3941*

Introduction

Salmonella is one of the leading causes of foodborne illnesses globally. Although the consumption of contaminated food has been identified as the major transmission pathway, direct or indirect contact with infected wild fauna, whether in captivity or in the natural environment, also plays a significant role in the epidemiology of salmonellosis [1,2]. Wild animals are important reservoirs of zoonotic pathogens, particularly *Salmonella* [1]. *Salmonella* is one of the major zoonotic pathogens transmitted from wildlife to humans due to its ubiquitous nature and remarkable ability to thrive under aFdverse environmental conditions [3,4]. *Salmonella* serovars are present in several species of wild animals in both captive and natural environment. However, epidemiological studies have been limited due to difficult access to the wild population [5]. Many wild animals may harbor *Salmonella* asymptotically, and shed the organism intermittently through faeces, thereby contaminate the environment. The bacterium may persist in harsh environmental conditions until the environment becomes favorable for growth and transmission to new susceptible host [3]. Modern zoological gardens are designed to mimic the natural environment of wild animals and have wide spaces for them to roam freely and display normal behaviours. However, this close simulation increases the likelihood of interaction between animals and visitors. Zoo visitors often approach fences or barriers and may come into contact with animals' faeces, resulting in possible transmission of zoonotic pathogens, including *Salmonella* [3,5]. *Salmonella* can persist in the environment for long period; hence, they can equally be transmitted to zoo visitors via contact with faecal-contaminated surfaces or exhibits [1,6].

Outbreaks of human salmonellosis associated with contact of visitors/zoo workers with wild animals in captivity have been documented in industrialized countries including those in Europe, Canada and Asia [3]. However, due to inadequate or absence of regular surveillance in Africa, there is no comprehensive data on the role of wild animals in the transmission pathway of salmonellosis. Moreover, the emergence of multidrug-resistant (MDR) *Salmonella* serovars from wild animals in captivity including reptiles and wild birds are available [2]. Therefore, the aim of the study is to determine the isolation rate and antimicrobial susceptibility profiles of *Salmonella* species recovered from captive wildlife at the University of Ilorin Zoological Garden, Kwara State, Nigeria.

Result

Rate of Isolation of Salmonella from Captive wildlife in Ilorin, Kwara State

In this study, a total of 191 samples were collected from different species of captive wild animals in the University of Ilorin zoological garden. Out of these, 19 samples (10.0 %) tested presumptive positive for *Salmonella* species. *Salmonella* was isolated from all classes of captive wildlife examined, though at different frequencies. The highest frequency of isolation was recorded among avian species (5.24 %), with geese showing the highest frequency (1.57 %) within this class. However, the differences in isolation rates within and between these groups were not statistically significant ($p > 0.05$). Only 1 (0.52 %) isolate each was obtained from reptiles and rodents. All isolates displayed typical biochemical characteristics consistent with *Salmonella* species: they were all Gram-negative rods, fermented glucose but not lactose, and reduced nitrate to nitrite (Table 1).

Distribution of Resistance phenotypes among Salmonella isolates from Captive Wildlife in Ilorin

The *Salmonella* isolates demonstrated varying frequencies of resistance to the antibiotics tested. The frequencies of resistance to ampicillin and erythromycin were 94.7 % and 89.5 % respectively. A single isolate (5.3 %) displayed resistance to cefotaxime, tetracycline, and neomycin while pan-susceptibility was observed to gentamicin by the isolates. The resistance rates to erythromycin (p= 0.042) and tetracycline (p= 0.035) were statistically significance among all the isolates. Similarly, resistance to ceftazidime was notably higher among isolates obtained from primate species (p = 0.002). Nine different resistance profiles were identified with ampicillin-erythromycin (AMP-E) phenotypes being the most prevalent (47.4 %). Three (5.8 %) isolates displayed multidrug-resistant (MDR) phenotypes, while 18 (94.7 %) isolates exhibited multi-antibiotic resistance indices ≥ 0.2 (fig. 1).

Discussion

The present study documented the occurrence of *Salmonella* among captive wildlife at the University of Ilorin Zoological Garden, highlighting the potential role of these animals as reservoir of *Salmonella*. The isolation rate of 10.0% observed in this study was higher than rates reported in India (3.1 %) [12], Trinidad (7 %) [13], and Tasmania (4.9 %) [14], but slightly lower than the 13.0 % reported among captive reptiles in Croatia [1].

Table 1.
Biochemical characterization of Salmonella isolates from captive wildlife at University of Ilorin Zoological Garden

Sample Source/Test	Ungulates	Carnivores	Avian	Reptiles	Rodents	Primates	Total
Gram reactions	Gram - rods	Gram - rods	Gram - rods	Gram - rods	Gram - rods	Gram - rods	
Urease	-	-	-	-	-	-	
Citrate	+	+	+	+	-	+	
H2S	+	+	+	+	+	+	
Glucose	+	+	+	+	+	+	
Lactose	-	-	-	-	-	-	
Sucrose	-	-	-	-	-	-	
Mannitol	+	+	+	+	+	+	
MR	+	+	+	+	+	+	
VP	-	-	-	-	-	-	
Indole	-	-	-	-	-	-	
ONPG	-	-	-	-	-	-	
Nitrate reduction	+	+	+	+	+	+	
No of positive	2.0	2.0	10.0	1.0	1.0	3.0	19.0
Detection	0.02	0.02	0.1	0.01	0.01	0.03	0.19

+= Positive, -=Negative, H2S= Hydrogen sulfide, VP=Voges Proskauer, MR= Methyl red, ONPG= ortho-nitrophenyl galactosidase

These variations could be attributed to differences in climatic conditions, management practices or sample sizes, as larger sample sizes increase the likelihood of recovering more isolates [15]. Among the different animal classes studied, the isolation rate was highest in captive wild birds, and this finding agrees with the previous studies identifying captive wild birds as major reservoirs of *Salmonella* within wildlife populations [5,11]. A high *Salmonella* rate among captive birds may indicate significant environmental contamination, increasing the risk of transmission to susceptible animals sharing the same environment. This could explain why *Salmonella* was isolated from all animal classes in the zoo. Inter-species transmission might also occur due to the close proximity of enclosures. Furthermore, flies could also serve as mechanical vectors, transmitting *Salmonella* from feacal droppings of infected animals to susceptible hosts via the feeds or water sources. This observation aligns with earlier findings suggesting that *Salmonella* survives longer in flies and beetles than many other zoonotic pathogens [4]. The presence of *Salmonella* in captive wild animals is of major zoonotic importance, as it poses a risk of human salmonellosis through direct contact with infected animals or indirectly via contaminated environment [1,3–5]. Interestingly, the frequency of *Salmonella* among captive reptiles (5.2 %) in this study was lower compared to previous reports, where reptiles are often considered highly susceptible to salmonellosis [5]. Such discrepancies may result from differences in geographic location, season, or sample size [4,5]. Lukac et al. [1] reported no *Salmonella* in captive reptiles in Croatia, suggesting that reptiles shed *Salmonella* intermittently, therefore the isolation rate at different times will vary based on the shedding rate at the period. The presence of *Salmonella* among carnivores (10.5 %) may be linked to their diet, as feeding on raw meat has been identified as a potential source of *Slmonella* [4]. In the current study, the frequency of isolation of *Salmonella* from primates is consistent with the

Class of wildlife	Source	No of isolate	Antibiotics									Patterns (N, %)	MARI
			CTX	CAZ	TE	E	FOX	CIP	N	CRO	AMP		
Equine	Horse	2				■	■				■	AMP-E-FOX (1, 5.3)	0.3
	Mule					■					■	AMP-E (3, 15.8)	0.2
Reptiles	Tortoise	1				■					■		
Rodents	Crested porcupine	1				■					■		
Carnivores	Hyena	2			■	■					■	AMP-E-TE (1, 5.3)	0.3
	Hyena					■					■	AMP-E (9, 47.4)	0.2
Avian	Emu	10				■					■		
	Geese					■					■		
	Geese					■					■		
	Geese					■					■		
	Pigeon					■					■		
	Pigeon					■					■		
	Crown dica					■					■		
	Peafowl					■					■		
	Guinea fowl					■	■	■		■	■	AMP-CIP-CRO-E-FOX (1, 5.3)	0.5
	Eagle					■	■	■			■	AMP-CIP-E-FOX (1, 5.3)	0.4
Primates	Monkey	3	■	■		■			■	■	■	AMP-CAZ-CRO-CTX (1, 5.3)	0.4
	Monkey			■							■	AMP-CAZ (1, 5.3)	0.2
	Monkey			■								CAZ (1, 5.3)	0.1
Total (%)		**19**	**1 (5.3)**	**3 (15.8)**	**1 (5.3)**	**17 (89.5)**	**3 (15.8)**	**2 (10.5)**	**1 (5.3)**	**2 (10.5)**	**18 (94.7)**	**MDR= 3 (15.8 %)**	
***P* value**			**0.34**	***0.002**	**0.11**	***0.042**	**0.067**	**0.85**	**0.34**	**0.80**	***0.035**		

Figure 1.
AMR profiles of Salmonella from captive wildlife in Ilorin. Black = resistance; White = Susceptible; Gray = MDR
CTX: Cefotaxime, CAZ: Ceftazidime, TE: Tetracycline, E: Erythromycin, FOX: Cefoxitin, CIP: Ciprofloxacin, N: Neomycin, CRO: Ceftriaxone, AMP: Ampicillin. * $p < 0.05$.

report of Gopee et al. [12] which reported that while it was rare for free-living wild primates to be infected, infection frequently occurs after they are placed in captivity. All isolates in this study showed distinct biochemical characteristics of *Salmonella* to biochemical reagents, consistent with previous reports [6]. Although biochemical characterizations are not commonly used for routine detection of *Salmonella* in the developed world because of their time consumption and low sensitivity, they are still the common methods available for routine diagnosis in developing countries [6,16].

Overall, the isolates displayed low resistance frequencies to most antimicrobials tested, except ampicillin (97.4 %) and erythromycin (89.5 %). These findings support previous studies reporting high rates of antimicrobial susceptibility among *Salmonella* isolates from wildlife [2,5]. For example, Farias et al. [2] reported that all *Salmonella* isolates from captive and exotic animal species in Ohio, USA, were pan-susceptible to all antimicrobial tested. The high resistance to ampicillin and erythromycin observed in this study could be due to selective pressure because of over-reliance on these antimicrobials in veterinary and animal production in the study area [14]. Although most isolates in the current study exhibited low resistance rates to antimicrobials, higher proportion of resistant isolates showed multidrug-resistant (MDR) phenotypes. This could be due to selective pressure on the antimicrobials in the environment [14,17]. In addition, a high proportion of the isolates have multiple antimicrobial resistance index (MARI) greater than 0.2, indicating that probably most of the isolates originated from high-risk sources and environments where overuse and abuse of antibiotics are common [10].

In conclusion, this study indicates that captive wildlife at the University of Ilorin zoological garden harbor *Salmonella* species at a prevalence rate of 10.0 %, with avian species showing the highest frequency of isolation. The isolates show low antimicrobial resistance levels, though some showed MDR phenotypes. These findings underscore the importance of continuous surveillance for foodborne pathogens among captive wild fauna to prevent cross species and zoonotic transmission.

Materials and Methods

Ethical Consideration

The study was approved by the Faculty of Veterinary Medicine, University of Ilorin the Ethical Review Committee with code UREC/FVM/15/32TA002.

Study area

The study was conducted at the University of Ilorin Zoological Garden located in Ilorin, the capital city of Kwara State, Nigeria. The zoo was originally established as a biological garden on the University's mini-campus in 1975 and was upgraded to a zoological garden in 1985 to support the teaching and research needs of the Department of Biological Sciences. The zoo is located near the main gate of the University, approximately between Lat. 80° 17" N & Long. 40° 82" [7].

Sample Collection

Fecal samples, were collected from overnight droppings of captive wild animals. For each animal, a sterile swab was inserted into the center of the fecal mass within the pen of each animal, as directed by the zoo attendants who helped restrain the animal when necessary. One sample was collected from each animal, on three separate visits, resulting in a total of 191 samples collected from ungulates (n= 25, 13.1 %), carnivores (n=31, 16.2 %), Avian (n= 83, 69.7 %), reptiles (n= 25, 13.1 %), rodents (n= 6, 3.1 %), and primates (n= 21, 11.0 %) (Table 2). All samples were transported to the Veterinary Microbiology Laboratory, University of Ilorin, within one hour of collection under a cold chain. Sample processing was initiated within 24 hours of their collection.

Sample Processing

The sample in swab stick was inoculated into 10 ml of peptone water (Oxoid, Hampshire, UK) and incubated at 35 ± 2°C for 22 ± 2 hours for pre-enrichment. Subsequently, enrichment was performed in Selenite-F broth (Oxoid, Hampshire, UK), prepared according to the manufacturer's instructions, by adding 1 mL of pre-enriched culture to 9 mL of the enrichment broth (ratio of 1:9) and then the mixture was incubated at 35 ± 2°C for 20 ± 2 hours [8]. After enrichment, samples were then selectively plated onto xylose lysine deoxycholate (XLD) agar (Oxoid, Hampshire, UK) and incubated at 35 ± 2°C for 20 ± 2 hours. Discrete pink colonies with black centers, suggestive of *Salmonella*, were sub-cultured on blood agar (Oxoid Ltd, Hampshire, UK) plates for purification and incubated at 35 ± 2°C for 20 ± 2 hours. The isolates were subjected to biochemical tests including IMVC tests (indole, methyl red, Voges Proskauer and citrate tests), urease test, triple sugar iron test and motility test. Presumptive *Salmonella* isolates were stored in Mueller Hinton broth containing 20 % glycerol at -20°C for further analysis.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the presumptive *Salmonella* isolates was performed using the Kirby-Bauer agar diffusion method as previously described [14]. The panel of antibiotics discs (Oxoid, Hampshire, UK) used in the assay contained following antibiotics and concentration: ceftriaxone (30 µg), erythromycin (10 µg), cefotaxime (30 µg), gentamicin (10 µg), neomycin (30 µg), ceftazidime (30 µg), tetracycline (30 µg), ampicillin (10 µg), cefoxitin (30 µg), and ciprofloxacin (5 µg).

Briefly; the presumptive isolates from stock were cultured on freshly prepared nutrient agar (Oxoid, Hampshire, UK) and incubated overnight at 35 ± 2 °C. Discrete colonies from the nutrient agar were inoculated into 10 ml of sterile normal saline in test

Akeem et al., IJVST 2025; Vol.17, No.4
DOI:10.22067/ijvst.2025.90724.1440

Table 2.
The rate of Isolation of *Salmonella* from captive wildlife at University of Ilorin Zoological Garden

Class of animal	Species of animal	No of sample (%)	Rate of isolation (%)
Ungulates	Horse	6 (3.1)	1 (0.52)
	Mule	2 (1.0)	1 (0.52)
	Donkey	10 (5.2)	0 (0.0)
	Camel	7 (3.7)	0 (0.0)
	Subtotal	25 (13.1)	2 (1.05)
Carnivores	Warthog	4 (2.1)	0 (0.0)
	Lion	4 (2.1)	0 (0.0)
	Hyena	10 (5.2)	2 (1.05)
	Leopard	5 (2.6)	0 (0.0)
	African civet cat	8 (4.2)	0 (0.0)
	Subtotal	31 (16.2)	2 (1.05)
Avian	Emu	4 (2.1)	1 (0.52)
	Geese	13 (6.8)	3 (1.57)
	Pigeon	11 (5.8)	2 (1.05)
	Crown dica	2 (1.0)	1 (0.52)
	Peafowl	7 (3.7)	1 (0.52)
	Guinea fowl	5 (2.6)	1 (0.52)
	Eagle	8 (4.2)	1 (0.52)
	Marabou stork	6 (3.1)	0 (0.0)
	Ostrich	6 (3.1)	0 (0.0)
	Duck	7 (3.7)	0 (0.0)
	Vulture	6 (3.1)	0 (0.0)
	White Indian fowl	4 (3.4)	0 (0.0)
	Black-crowned crane	4 (3.4)	0 (0.0)
	Subtotal	83 (69.7)	10 (5.24)
Reptiles	Tortoise	6 (3.1)	1 (0.52)
	Crocodile	9 (4.7)	0 (0.0)
	Puff adder	4 (3.4)	0 (0.0)
	Royal python	6 (3.1)	0 (0.0)
	Subtotal	25 (13.1)	1 (0.52)
Rodents	Crested porcupine	6 (3.1)	1 (0.52)
	Subtotal	6 (3.1)	1 (0.52)
Primates	Monkey	16 (8.4)	3 (1.57)
	Baboon	5 (2.6)	0 (0.0)
	Subtotal	21 (11.0)	3 (1.57)
	Total	**191 (100.0)**	**19 (10.0)**

tubes using a sterile wire loop. The turbidity of the inoculum was adjusted to 0.5 McFarland standards using a Nephelometer (Oxoid, Hampshire, UK). The inoculum was poured on the Mueller Hinton agar plate (Oxoid, Hampshire, UK) and it was uniformly spread until the surface of the agar plate was covered by the inoculum. Excess inoculum was discarded after 30 seconds. Plates were partly left open for 3-5 minutes on the sterilised working bench until they dried. Antibiotic sensitivity discs were dispensed on each plate using a disc dispenser (Oxoid, Hampshire, UK). Plates were then incubated at 35 ± 2°C for 18 ± 2 hours. The inhibition zones of each antimicrobial were measured using a vernier calliper (Hi-Media, Mumbai, India) and recorded according to CLSI standards [9,10]. *E. coli* ATCC 25922 was used as a control strain. The multiple antimicrobial resistance index (MARI) was determined according to standard methods as previously described [11].

Statistical Analysis

The data were computed in a Microsoft Excel 2019 database. The overall isolation rate and the frequency of *Salmonella* from each wild animal sampled was determined. Statistical estimates were made using Graphpad Prism statistical package, San Diego, Califonia, U.S.A (www.Graphpad.Com) at confidence interval of 95 %. Probability values less than 0.05 ($p < 0.05$) were considered significant. Chi-square was used to determine the level of significance in the rates of isolation between and within different classes of wild animals under the study.

Authors' Contributions

Conceptualization and Study design: AOA and RAI. Material preparation, data collection, and analysis: KAA, SAA, ALF, SBA AA, and AMI. Manuscript draft, editing, and reviewing: AOA, RAI, KAA, SAA, ALF, SBA, AA, and AMI. All authors read and approved the final manuscript.

Acknowledgements

The authors wish to acknowledge the technical support from the technological staff of the Department of Veterinary Microbiology, University of Ilorin for the technical assistance during the work.

Competing Interests

The authors declare that there is no conflict of interest.

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How to cite this article
Akeem AO, Abdulhameed KA, Ahmad SA, Fateema AL, Abdulkareem SB, Abdulfatai A, et al. EIsolation Rate and Antimicrobial Profiles of *Salmonella* from Captive Wild Animals at University of Ilorin Zoological Garden, Kwara State, Nigeria. Iran J Vet Sci Technol. 2025; 17(4): 18-25.
DOI: https://doi.org/**10.22067/ijvst.2025.90724.1440**
URL:https://ijvst.um.ac.ir/article_47084.html