Research Article

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

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Abstract

Salmonellosis is a zoonotic disease of global epidemiology. The role of captive wildlife in the epidemiology of salmonellosis is elusive, especially in developing countries. This study aimed to determine the rate of isolation and antimicrobial profiles of *Salmonella* from captive wildlife at the University of Ilorin Zoological Garden. In a cross-sectional study, 191 faecal samples collected from different animals were subjected to standard bacteriological procedures. Antimicrobial sensitivity testing was conducted on the isolates per the Kirby-Bauer disk diffusion technique. Nineteen (10.0 %) samples were positive for *Salmonella*. The frequencies of isolation varied among the different classes of wild animals sampled, with the highest isolation rate (5.24 %) from avian species. However, the differences in the isolation rates within and between the different classes of were not statistically significant ($P > 0.05$). The isolates generally showed a low level of antimicrobial resistance to the majority of the antibiotics tested, except ampicillin and erythromycin, to which 94.7 % and 89.5 % of the isolates displayed resistant phenotypes, respectively. The resistance rates to erythromycin and tetracycline were statistically significant among all the isolates ($P = 0.042$) and ($P = 0.035$), respectively. Similarly, resistance to ceftazidime is prominent among the primate species sampled ($P = 0.002$). Nine different resistance profiles were detected, and 15.8 % of resistant isolates displayed multidrug-resistant (MDR) phenotypes. Eighteen 94.7 % of the *Salmonella* isolates possess multi-antibiotic resistance (MAR) index ≥ 0.2. Continuous monitoring is essential to determine the zoo's primary source of infection and control environmental contamination by MDR zoonotic pathogens.

Abbreviations

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

Introduction

Salmonella is one of the leading causes of foodborne illnesses globally. Even though consumption of contaminated food has been identified as the major transmission pathway, contact with/consumption of infected wild fauna, either in captivity or in the natural environment, plays a significant role in the epidemiology of salmonellosis [1,2]. Wild animals are important reservoirs of zoonotic pathogens, especially *Salmonella* [1]. *Salmonella* is one of the major zoonotic pathogens often contracted from wild animals because it is ubiquitous and can thrive in adversity [3,4]. *Salmonella* serovars are present in several species of wild animals in the captive or natural environment. Still, epidemiological studies have been limited due to difficult access to the wild population [5]. Many wild animals may harbour *Salmonella* infections asymptotically; thus, apparently healthy animals may excrete *Salmonella* in their faeces, leading to environmental contamination. This bacterium may persist under adverse environmental conditions until the environment becomes favourable for its growth and infection of a new susceptible host [3]. Nowadays, zoos are designed to mimic the natural environment of wild animals and have wide spaces for them to roam freely and display normal behaviours. The visitors sometimes come close to the fence around the animals and may touch them. Hence, they can come in 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 nations including 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. Reports 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* from captive wildlife at the University of Ilorin Zoological Garden.

Results

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

In this study, out of the 191 samples collected from different species of captive wildlife in the University of Ilorin zoological garden, 19.0 (10.0 %) showed presumptive positive for *Salmonella*. *Salmonella* was isolated from all the different classes of captive wildlife sampled at different frequencies with the maximum frequency of isolation obtained from avian species (5.24 %) (geese have the highest frequency (1.57 %) among the avian species). However, the differences in the isolation rates within and between the different classes of were not statistically significant ($P > 0.05$). Only 1 (0.52 %) isolate was obtained from reptiles and rodents sampled. All the isolates displayed typical biochemical characteristics of *Salmonella*. They were all Gram-negative rods, fermented glucose but not lactose while all reduced nitrate to nitrite (Table 2).

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

Varying frequencies of resistance were obtained for the antibiotics tested. The frequencies of resistance to ampicillin and erythromycin were 94.7 % and 89.5 % respectively. One (5.3 %) of the isolates displayed resistance phenotype to cefotaxime, tetracycline, and neomycin while pan-susceptibility was observed to gentamicin by the isolates. The resistance rates to erythromycin and tetracycline were statistically significance among all the isolates ($P = 0.042$) and ($P = 0.035$) respectively. Similarly, resistance to ceftazidime is prominent among the primate species sampled ($P = 0.002$). Nine different resistance profiles were detected with ampicillin-erythromycin (AMP-E) phenotypes being the most predominant (47.4 %). Three

(5.8 %) of the resistant isolates displayed multidrug-resistant (MDR) phenotypes. Eighteen (94.7 %) isolates possess multi-antibiotic resistance indices ≥ 0.2 (fig. 1).

Discussion

The present study documented the occurrence of *Salmonella* among captive wildlife in the University of Ilorin Zoological Garden and showed the importance of captive wildlife as a reservoir of *Salmonella*. The rate (10.0 %) of *Salmonella* isolation in the current study is high compared to 3.1 % reported in India [12], 7 % in Trinidad [13], and 4.9 % in Tasmania [14]. It is, however, slightly lower compared to the reports of *Salmonella* from captive reptiles (13.0 %) in Croatia [1]. These variations might be due to differences in climatic conditions, management practices or differences in the sample size since sample size determines the probability of obtaining more isolates on culture as previously reported [15]. Considering the classes of wild animals in the studied zoo, the isolation rate was highest among the captive wild birds sampled and this supports the previous studies which reported captive wild birds as major reservoirs of *Salmonella* among the wild fauna [5,11]. The implication of a high *Salmonella* rate among the captive birds is that there is a high likelihood of environmental contamination and therefore, *Salmonella* transmission to susceptible hosts sharing the same environment. This might be the reason why *Salmonella* was isolated among all the classes of captive wildlife in the studied garden. The presence of *Salmonella* among all the classes of animals in the zoological garden studied might be due to inter-species transmission because of the proximity of the animals. Flies could also serve as vectors transmitting *Salmonella* from feacal droppings of infected animals to susceptible hosts via the feeds or water as *Salmonella* has been reported to survive better in flies and beetles than other zoonotic pathogens [4]. The presence of *Salmonella* among captive wild animals is of zoonotic significance as it may serve as a source of human salmonellosis directly through contact with infected hosts or indirectly via contaminated environment by faeces of infected animals [1,3–5]. The frequency (5.2 %) of

Salmonella among captive reptiles was low compared to previous reports which documented reptiles were highly susceptible to salmonellosis [5]. The differences could be due to differences in geographic location, season of study and sample size as previously reported [4,5]. Lukac *et al*. [1] reported no *Salmonella* from captive reptiles in Croatia, it was therefore postulated that *Salmonella* is shed intermittently in reptiles and therefore, the isolation rate at different times will vary based on the shedding rate at the period. The presence of *Salmonella* among the sampled carnivores (10.5 %) could be due to feeding these animals with raw meat which could be contaminated with *Salmonella* as reported [4]. In the current study, the frequency of isolation of *Salmonella* from primates was in tandem with Gopee *et al*. [12] which reported that it was rare for free-living wild primates to be infected at the time of capture but they frequently become infected in captivity. All the isolates showed distinct biochemical characteristics of *Salmonella* to biochemical reagents and this is in tandem 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].

Generally, the isolates displayed low frequencies of resistance to all antimicrobials tested, with the exception of ampicillin and erythromycin, to which 97.4 % and 89.5 % of the isolates exhibited resistance, respectively. The results of this study corroborate previous studies that most isolates from wildlife showed high rates of antimicrobial susceptibility [2,5]. Farias *et al.* [2] reported that all *Salmonella* from captive wild fauna and exotic animal species in Ohio, USA were pan-susceptible to all antimicrobial tested. The resistance to ampicillin and erythromycin in the studied animals could be due to selective pressure because of over-reliance on these antimicrobials in veterinary and animal production in the study area [14]. Even though the isolates in the current study exhibited low resistance rates to antimicrobials, higher proportions of the resistant isolates showed multidrug-resistant (MDR) phenotypes. This may

likely be due to selective pressure on the antimicrobials in the environment as previously reported [14,17]. In addition, a high proportion of the isolates have multiple antimicrobial resistance index (MARI) greater than 0.2. MARI >0.2 indicates that most of the isolates probably originate 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 harbours a *Salmonella* species at the rate of 10.0 %, with the highest frequency of isolation from avian species. The isolates show low antimicrobial resistance with the majority of the resistant isolates showing MDR phenotypes. Further study is necessary to characterize the isolates genotypically. Continuous surveillance for foodborne pathogens among captive wild fauna is recommended to monitor the transmission of the pathogens among the different species of animals and humans.

Materials and Methods

Study area

The study area was University of Ilorin Zoological Garden. The zoo was first established as the biological garden at the mini-campus of the University in 1975 and upgraded to a zoological garden in 1985 to compensate the University's Biological Sciences Departments in teaching and research. The zoo is located approximately between Lat. 80^0 17' N & Long. 40^0 82" E near the main gate of the University. Ilorin, the capital of Kwara state, Nigeria [7].

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.

Sample Collection

Faecal samples, from overnight voided faeces, were collected from each animal by dipping a sterile swab inside the centre of the faecal mass in the pen of each animal (the zoo attendant

would have directed the animal to restraints corner). One sample was collected from each animal. The zoo was visited three times with 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 1). All the samples were shipped under a cold chain, within one hour, to the University of Ilorin Veterinary Microbiology Laboratory for analysis. The processing of the samples occurs within 24 hours of their collection.

Sample Processing

The sample in swab stick was inoculated in 10 ml of peptone water (Oxoid, Hampshire, UK) and incubated at 35 ± 2 ^{0}C for 22 ± 2 hours. Selenite-F broth (Oxoid, Hampshire, UK) was prepared according to the manufacturer's instructions. Pre-enriched samples were enriched in the Selenite-F broth at the ratio of 1:9 and incubated at 35 ± 2 ^{0}C for 20 ± 2 hours [8]. The enriched samples were then selectively plated on xylose lysine deoxycholate agar, XLD (Oxoid, Hampshire, UK) and incubated at 35 ± 2 ^{0}C for 20 ± 2 hours. Discrete pinkish colonies with dark centres suggestive of *Salmonella* were sub-cultured on blood agar plates for purification (Oxoid Ltd, Hampshire, UK) at 35 ± 2 ^{0}C for 20±2 hours. The isolates were subjected to biochemical tests including IMVC (indole, methyl red, Voges Proskauer and citrate tests), urease, triple sugar iron and motility test were also done. The presumptive *Salmonella* isolates were stored in Mueller Hinton broth containing 20 % glycerol at -20 ^{0}C for further analysis.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was done on the presumptive *Salmonella* isolates adopting the Kirby-Bauer agar diffusion method as previously described [14]. The panel of antibiotics discs (Oxoid, Hampshire, UK) used in the assay have the following antibiotics with the concentration in parentheses: 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 ^{0}C. Discrete colonies from the nutrient agar were inoculated into 10 ml of sterile normal saline in test 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. The 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). The plates were then incubated at 35 ± 2 ^{0}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. 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 rate of isolation from overall samples as well as 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.

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Table 1.
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)**

Table 2.
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	+	+	+	+	-	+	
H_2S	+	+	+	+	+	+	
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, H_2S= Hydrogen sulfide, VP=Voges Proskauer, MR= Methyl red, ONPG= ortho-nitrophenyl galactosidase

Class of wildlife	Source	No of isolate	Antibiotics									
			CTX	CAZ	TE	E	FOX	CIP	N	CRO	AMP	Pa
Equine	Horse	2										A
	Mule											A
Reptiles	Tortoise	1										
Rodents	Crested porcupine	1										
Carnivores	Hyena	2										A
	Hyena											A
Avian	Emu	10										
	Geese											
	Geese											
	Geese											
	Pigeon											
	Pigeon											
	Crown dica											
	Peafowl											
	Guinea fowl											A (1
	Eagle											A
Primates	Monkey	3										A 5.
	Monkey											A
	Monkey											C
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)**	
***P* value**			**0.34**	***0.002**	**0.11**	***0.042**	**0.067**	**0.85**	**0.34**	**0.80**	***0.035**	

Fig 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. * $P < 0.05$.