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Detection and Antibiotic-resistance of *Salmonella* species and *Escherichia coli* from Selected Captive Animals in Ogba Zoological Garden

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ABSTRACT

Animals kept at the zoo are customarily raised in confinement, captured in the wild or acquired from other facilities. They have been reported to be connected with *Escherichia coli* and *Salmonella* infections with a significant hazard to public health. This study was designed to elucidate antimicrobial susceptibility profile of *Salmonella* species and *Escherichia coli* isolated from captive animals in Ogba Zoological Garden. A total of 60 faecal samples were randomly collected from captive animals between April and June 2017 and analyzed using standard culture-based techniques. Antimicrobial susceptibility profile of the isolates was carried out using the disk diffusion method. The mean cell density was $4.0 \times 10^8 \pm 0.60$ cfu/g for heterotrophic bacteria; $7.9 \times 10^5 \pm 1.28$ cfu/g for *Escherichia coli* and $1.0 \times 10^6 \pm 2.81$ cfu/g for *Salmonella* species. All faecal samples examined in this study were positive for *Salmonella* species and *E. coli*. A total of 38 *E. coli* and 44 *Salmonella* species were selected and subjected to antibiogram characterization. Antibiogram findings revealed that 38/38 (100%) of the *E. coli* isolates were resistant to 3 antibiotics (cefepime, meropenem, tetracycline) which belongs to 3 antimicrobial class (cephems, carbapenems and tetracyclines) with multiple antibiotic resistant index of 0.25; while 44/44 (100.0%) of the *Salmonella* isolates were resistant to 3 antibiotics (cefepime, ertapenem, tetracycline) which belongs to 3 antimicrobial class (cephems, carbapenems and tetracyclines) with multiple antibiotic resistant index of 0.27. However, 36/38 (94.7%) and 30/38 (78.9%) of the *E. coli* isolates were sensitive to cefotaxime and ceftriaxone respectively. Also, 12/44 (27.3%) and 14/44 (31.8%) of the *Salmonella* isolates were sensitive to ceftriaxone and gentamycin respectively. The presence of these potential pathogens in the faeces of captive animals coupled with their multidrug resistance potential call for public health concern as this could be a route of transfer of multidrug-resistant pathogens in the environment.

Keywords: Captive animals, Zoo, *Salmonella*, *Escherichia coli*, Multidrug-resistant, Public health

Introduction

Interactions with animals afford numerous benefits to adults and children via entertainment and education. Many health benefits of the animal-human interface have been acknowledged and comprise lowered blood pressure and reduced anxiety (Dunn *et al.*, 2015). Contact with catchment animals occurs in a variety of public settings, such as petting

zoos, rodeos, farm tours or visits, livestock exhibitions, and state or county agricultural fairs (Pickering *et al.*, 2008; Conrad *et al.*, 2017). Despite the numerous benefits of public catchment centres, there are significant risks involved if proper hygiene measures are not practised. Of significant importance are zoonoses (or zoonotic diseases), which are infections that can be disseminated amongst humans and animals.

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Page | 3072

These disease-causing agents may be disseminated in animal faeces and disseminated to humans via faecal-oral routes and the environment. The study of disease-causing microorganisms from animal populations kept in the Zoo is crucial for execution of programs for prevention and control as well as surveillance of infectious diseases, for the advancement of animal and public health policies.

Studies have shown that infants and children less than 5 years are at the apex risk of obtaining zoonotic pathogens from animals (Dunn *et al.*, 2015). Often times, this is ascribed to attraction to or inquisitiveness about animals, poor hygienic practices, and an immune-compromised or inadequate immune system (Pickering *et al.*, 2008; Dunn *et al.*, 2015). Often times, the symptoms and clinical expressions have been recounted to be more morbid in young children and infants (Pickering *et al.*, 2008). In some scenarios, the zoonotic illnesses are avoidable via improved hygiene (Erdozain *et al.*, 2015; Conrad *et al.*, 2017). However, the symptomatology and consequences of such expressions have been reported. Individuals irrespective of their age with secondary or primary immune-deficiency are exposed to even more severe diseases, with the elderly and antenatal women not excluded (Pickering *et al.*, 2008). Most often, zoonotic infections are microbial complications which emanate from the gastrointestinal tract, with morbid to fatal outcomes ranging from abdominal cramps, vomiting and diarrhoea to bloody diarrhoea, kidney failure, and, in severe scenarios, death (Dunn *et al.*, 2015).

Salmonella species have been described to produce asymptomatic as well as symptomatic clinical manifestations in humans and animals, with symptoms which include fever, diarrhoea, abortion, vomiting, osteomyelitis and intermittently death (Stevens *et al.*, 2009). Also, *Escherichia coli* are commensal bacteria that are usually non-pathogenic and reside within the large intestine of warm-blooded animals and human (Beshiru *et al.*, 2016; Marchant *et al.*, 2016). Additionally, some strains of *E. coli* and *Salmonella* have been reported as a global zoonotic pathogen that can result in bloody diarrhoea, haemolytic uremic syndrome and hemorrhagic colitis in humans (Chaudhuri and Henderson, 2012). *Salmonella* species have been identified from captive animals with asymptomatic manifestations (Stirling *et al.*, 2008; Jardine *et al.*, 2011), and has also resulted in disease outbreaks with increased death rates (Marchant *et al.*, 2016). In the case of *E. coli*, reports in captive animals have revealed variable prevalence in the range of 0.1% to 50.8%, probably as a result of the different detection/isolation procedures and species of animals being studied, though asymptomatic manifestation is always described (Stirling *et al.*, 2008).

Antibiotic-resistant bacteria are significantly important to human health, but the reservoirs of resistance determinants are poorly understood. The genesis of antimicrobial resistance in catchment animals is significant to human health due to the cumulative importance of zoonotic diseases and the necessity for predicting the development of resistant pathogens (Radhouani *et al.*, 2014). Proximity to human activities impacts the antibiotic resistance profiles of bacteria in captive mammals, which reside in densely populated microbial habitats where antibiotics select for resistance. Though numerous bacterial species are significant in terms of multidrug resistance in veterinary and human medicine, *E. coli* and *Salmonella* are considered as key bacterial pathogens to monitor the advancement of multiple drug-resistant bacteria in captive animals (Radhouani *et al.*, 2014).

Salmonella cases emanating from humans have been tied to direct or indirect interaction with animal sources in several independent reports (Hernandez *et al.*, 2012; Hauser *et al.*, 2012; Lawson *et al.*, 2014). The majority of those reports relied chiefly on post-outbreak surveys with few utilizing molecular typing to investigate similarities between human and animal isolates (Hernandez *et al.*, 2012). Both *E. coli* and/or *Salmonella* species have been detected from several captive animal species in Chile (Marchant *et al.*, 2016), Malaysia (Saleha *et al.*, 2015), USA (Hernandez *et al.*, 2016), Brazil (Suphoronski *et al.*, 2015), Iran (Koochakzadeh *et al.*, 2015), Grenada (Amadi *et al.*, 2015), Spain (Núñez-Díaz *et al.*, 2017), Japan (Kabeya *et al.*, 2017), Egypt (Mahmoud, 2015), and Sri Lanka (Kulasooriya *et al.*, 2016) with limited information from Nigeria. This study was carried out to elucidate the antimicrobial susceptibility profiles of *Salmonella* species and *E. coli* in captive animals from Ogba zoological Garden, Benin City, Nigeria.

MATERIALS AND METHODS

Study Area

Ogba Zoo was established in 1965 in a forest reserve in Ogba District, four km from the city centre. Ogba Zoo (also known as Ogba Zoo and Nature Park) is an extensive state-owned multi-purpose recreational centre in 750 Acres of land as well as Urban Park with tourist resort development and Biological Garden. It is located between Oko and Ogba district with coordinate's 6°17'20" N 5°35'16" E; 5 km Airport road, Benin City, Nigeria. The Ogba Zoo is home to a number of local and wild species of animals including lions, primates, rock pythons, giant tortoise, antelope and equine species to mention a few. The Zoo opens at 9 am and closes at 6 pm. In addition, the Ogba Zoo and Nature

Park also have an exclusive event ground with open access and robust facilities that accommodates an average of 1, 500 persons with free space for side activities.

Sample Collection

Page |

3073

A total of 60 faecal specimens were randomly collected from captive animals (Guinea fowl, Chimpanzee, Spotted hyena, Red Potas and Baboon) between April and June 2017. Faecal samples were collected from different points at the catchment center with the aid of sterile glass container, labeled appropriately and transported to the Applied Microbial Processes and Environmental Health Research Group Laboratory at the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City in cold ice pack and analyzed within 4 h after collection.

Isolation and identification of *E. coli* and *Salmonella* species

Samples were serially diluted and spread plated into separate Nutrient agar (NA) (Lab M, UK) for heterotrophic bacteria enumeration; Hektoen Enteric agar (HEA) (Lab M, UK) for *Salmonella* and Chromocult Coliform agar (CCA) (Merck, Germany) for *E. coli* and incubated at a $37\pm 2^\circ\text{C}$ for 18-24 h. After incubation, discrete colonies were counted and expressed in colony forming unit per gram. The colour of *Salmonella* sp. on the HEA agar plate was observed as green with dark centres as well as completely black isolates, while the colour of *E. coli* on CCA was observed as purple to violet colour for presumptive identification and enumeration. Enrichment using Selenite F-Broth for *Salmonella* species (Lab M, UK) and Tryptone Soy Broth (Merck, Germany) for *E. coli* was incubated for 18-24 h at 37°C . Thereafter, streak plate technique was employed to streak directly on HEA and CCA plate and incubated for another 18-24 h at 37°C (Igbinosa, 2015). Distinct colonies were repeatedly sub-cultured, purified on nutrient agar and stored at refrigeration temperature for further analysis. Gram reaction with 3% KOH test; oxidase and catalase assessments were carried out. Gram-negative, catalase-positive and oxidase negative isolates were suspended in normal saline (0.8%) and turbidity adjusted to 0.5 McFarland standard. The bacteria suspension was inoculated onto API 20E strips (bioMérieux, Marcy-L'Étoile, France) following user' manual, strips were incubated for 24 h and read. The results were interpreted using API 20E software).

Antibiotic Susceptibility Test

The *Salmonella* and *E. coli* isolates that were positively identified using the culture-based method were subjected to antibiogram characterization. All the bacterial isolates were assessed for sensitivity or resistance to standard antibiotics using the Kirby-Bauer test method. For the disc diffusion assay, bacteria were grown between 18 and 24 h on Mueller-Hinton agar, harvested and then suspended in 0.85 % saline solution adjusted to a 0.5 McFarland turbidity standard. The inoculum was streaked onto plates of Mueller-Hinton agar (Merck, Germany) using a sterile cotton swab and impregnated with appropriate antibiotics. The outcomes were documented after incubating for 24 h at 37°C . Commercially available antibiotics disc (Mast Diagnostics, Merseyside, United Kingdom) were used to elucidate the resistance forms of the isolates against ten different antibiotics (1 dose/disc) grouped into seven different classes of antibiotics. The width zone of inhibition surrounding each disc was measured and interpreted as resistance (R), intermediate (I) or sensitive (S) in accordance with the recommended standard established by the Clinical Laboratory Standard Institute (2017).

Statistical Analysis

All data were statistically analyzed using the statistical package IBM SPSS Statistics, version 21.0. All values in triplicates were analyzed using descriptive statistics and expressed as the mean \pm standard deviation of the mean.

RESULTS

The population density of the bacterial isolates

The mean population density of the bacteria isolated from the faeces of the captive animals is presented in Table 1 below. The total heterotrophic bacteria ranged from 3.0×10^7 to 4.5×10^{10} cfu/g with a mean cell density of $4.0 \times 10^8 \pm 0.60$ cfu/g. The *Escherichia coli* count ranged from 6.4×10^3 to 3.5×10^6 cfu/g with a mean of $7.9 \times 10^5 \pm 1.28$ cfu/g. The *Salmonella* species count ranged from 2.0×10^2 to 8.0×10^6 cfu/g with a mean of $1.0 \times 10^6 \pm 2.81$ cfu/g. A significant difference was observed in the population densities expressed ($p < 0.05$). Findings from the selected captive animals studied [Guinea fowl (14), Chimpanzee (10), Spotted hyena (13), Red potas (13) and Baboon (10)] revealed that all samples harboured *Salmonella* species and *E. coli* respectively culminating in 100% prevalence.

Identification of *Salmonella* and *Escherichia coli* isolates from captive animals

The sum of 44 *Salmonella* and 28 *E. coli* were recovered from the faecal samples of captive animals. The identification was established on the basis of

biochemical characterization. The distribution of the *Salmonella* and *Escherichia coli* isolates in the different captive animals is as shown in Table 2.

Antimicrobial susceptibility profile of the bacterial isolates

Page | 3074
The resistant profile of 38 selected and purified *E. coli* against 12 antibiotics belonging to 7 groups of antimicrobials (Table 3) revealed that 38/38 (100%) were resistant to cefepime (Cephems), meropenem (Carbapenems), and tetracycline (Tetracyclines). In addition, 36/38 (94.7%) were resistant to cefuroxime (Cephems), 32/38 (84.2%) were resistant to ceftazidime (Cephems), 22/38 (57.9%) were resistant to gentamicin (Aminoglycosides), with 20/38 (52.6%) resistant to aztreonam (Monobactam). Likewise, 36/38 (94.7%) were sensitive to cefotaxime (Cephems), 30/38 (78.9%) were sensitive to ceftriaxone (Cephems), 24/38 (63.1%) were sensitive to ciprofloxacin (Fluoroquinolone), while 20/38 (52.6%) were sensitive to piperacillin (Penicillins).

The resistant profile of 44 selected and purified *Salmonella* species (Table 3) on 11 antibiotics belonging to 7 groups of antimicrobials revealed that 44/44 (100%) were resistant to cefepime (Cephems), ertapenem (Carbapenems), and tetracycline (Tetracyclines). Furthermore, 42/44 (95.5%) were resistant to cefotaxime (Cephems), 34/44 (77.3%) were resistant to aztreonam (Monobactam), 32/44 (72.7%) were resistant to imipenem (Carbapenems), while 28/44 (63.6%) were resistant to ciprofloxacin (Fluoroquinolone). Contrarily, 14/44 (31.8%) were sensitive to gentamicin (Aminoglycosides), and 12/44 (27.3) to ceftriaxone (Cephems).

Multidrug-resistant profile of the bacterial isolates

Multidrug-resistant profile revealed that 3/38 (7.89%) of the *E. coli* isolates were resistant to 9 antibiotics (CPM^R, CAZ^R, CXM^R, ATM^R, MEM^R, IMI^R, GEN^R, TET^R, CIP^R) which belongs to 6 antimicrobial class with multiple antibiotic resistant index of 0.75. More so, 38/38 (100%) were resistant to 3 antibiotics (CPM^R, MEM^R, TET^R) which belongs to 3 antimicrobial class with multiple antibiotic resistant index of 0.25 (Table 4a).

Similarly, the multidrug-resistant profile of the *Salmonella* isolates revealed that 9/44 (20.46%) were resistant to 10 antibiotics (CTX^R, CPM^R, CTR^R, ATM^R, PIP^R, IMI^R, ETP^R, GEN^R, TET^R, CIP^R) which belongs to 7 antimicrobial class with multiple antibiotic resistant index of 0.91. More so, 44/44 (100.0%) were resistant to 3 antibiotics (CPM^R, ETP^R, TET^R) which belongs to 3 antimicrobial class with multiple antibiotic resistant index of 0.27 (Table 4b).

Table 1. The population density of the bacterial isolates

Population density	Minimum cfu/g	Maximum cfu/g	Mean ± SD cfu/g
Heterotrophic bacteria	3.0×10^7	4.5×10^{10}	$4.0 \times 10^8 \pm 0.60$
<i>Escherichia coli</i>	6.4×10^3	3.5×10^6	$7.9 \times 10^5 \pm 1.28$
<i>Salmonella</i> species	2.0×10^2	8.0×10^6	$1.0 \times 10^6 \pm 2.81$
p-value			0.001

Legend: Values presented in the table represent the mean ± standard deviation from the population density the entire samples.

Table 2. Distribution of *Salmonella* species and *E. coli* in faecal samples of captive animals

Captive animals	<i>Salmonella</i> sp.	<i>E. coli</i>
Guinea fowl	18 (40.9%)	13 (46.42%)
Chimpanzee	5 (11.3%)	7 (25%)
Spotted hyena	3 (6.8%)	5 (17.9%)
Red potas	7 (15.93)	4 (14.2%)
Baboon	11 (25%)	9 (32%)

Table 3. Antimicrobial susceptibility profile of *Salmonella* species and *Escherichia coli* recovered from fecal specimen

Antimicrobial class	Antibiotics	<i>Escherichia coli</i> n=38			<i>Salmonella</i> species n=44		
		R	I	S	R	I	S
Cephems	Cefotaxime 30µg	0 (0)	2 (5.3)	36 (94.7)	42 (95.5)	2 (4.5)	0 (0)
	Cefepime 30µg	38 (100)	0 (0)	0 (0)	44 (100)	0 (0)	0 (0)
	Ceftriaxone 30µg	0 (0)	8 (21.1)	30 (78.9)	10 (22.7)	22 (50)	12 (27.3)
	Ceftazidime 30µg	32 (84.2)	6 (15.7)	0 (0)	ND	ND	ND
	Cefuroxime 30µg	36 (94.7)	2 (5.3)	0 (0)	ND	ND	ND
Monobactam	Aztreonam 30µg	20 (52.6)	10 (26.3)	8 (21.1)	34 (77.3)	10 (22.7)	0 (0)
Penicillins	Piperacillin 100µg	0 (0)	18 (47.3)	20 (52.6)	16 (36.4)	28 (63.6)	0 (0)
Carbapenems	Meropenem 10µg	38 (100)	0 (0)	0 (0)	ND	ND	ND
	Imipenem 10µg	4 (10.5)	34 (89.5)	0 (0)	32 (72.7)	12 (27.3)	0 (0)
	Ertapenem 10µg	ND	ND	ND	44 (100)	0 (0)	0 (0)
Aminoglycosides	Gentamicin 10µg	22 (57.9)	16 (42.1)	0 (0)	10 (22.7)	20 (45.5)	14 (31.8)
	Kanamycin 30µg	ND	ND	ND	0 (0)	42 (95.5)	2 (4.5)
Tetracyclines	Tetracycline 30µg	38 (100)	0 (0)	0 (0)	44 (100)	0 (0)	0 (0)
Fluoroquinolone	Ciprofloxacin 5µg	8 (21.1)	6 (15.7)	24 (63.1)	28 (63.6)	14 (31.8)	2 (4.5)

Legend: R-Resistant; I- Intermediate; S- Sensitive; ND-Not determined

Table 4a. Multidrug-resistant profile of *E. coli* isolates from captive animals

Antimicrobial class	Antibiotics	Resistant phenotype	Number of isolates n=38	Multiple antibiotic-resistant index
6	9	CPM ^R , CAZ ^R , CXM ^R , ATM ^R , MEM ^R , IMI ^R , GEN ^R , TET ^R , CIP ^R	3 (7.89)	0.75
6	8	CPM ^R , CAZ ^R , CXM ^R , ATM ^R , MEM ^R , GEN ^R , TET ^R , CIP ^R	6 (15.79)	0.66
5	7	CPM ^R , CAZ ^R , CXM ^R , ATM ^R , MEM ^R , GEN ^R , TET ^R	17 (44.74)	0.58
3	5	CPM ^R , CAZ ^R , CXM ^R , MEM ^R , TET ^R	30 (78.95)	0.42
3	4	CPM ^R , CXM ^R , MEM ^R , TET ^R	35 (92.11)	0.33
3	3	CPM ^R , MEM ^R , TET ^R	38 (100)	0.25

Legend: CTX: Cefotaxime, CPM: Cefepime, CTR: Ceftriaxone, CAZ: Ceftazidime, CXM: Cefuroxime, ATM: Aztreonam, PIP: Piperacillin, MEM: Meropenem, IMI: Imipenem, GEN: Gentamicin, TET: Tetracycline, CIP: Ciprofloxacin.

Table 4b. Multidrug-resistant profile of *Salmonella* species from captive animals

Antimicrobial class	Antibiotics	Resistant phenotype	Number of isolates n=44	Multiple antibiotic resistant index
7	10	CTX ^R , CPM ^R , CTR ^R , ATM ^R , PIP ^R , IMI ^R , ETP ^R , GEN ^R , TET ^R , CIP ^R	9 (20.46)	0.91
6	8	CTX ^R , CPM ^R , ATM ^R , PIP ^R , IMI ^R , ETP ^R , TET ^R , CIP ^R	14 (31.82)	0.73
5	7	CTX ^R , CPM ^R , ATM ^R , IMI ^R , ETP ^R , TET ^R , CIP ^R	22 (50.0)	0.64
4	6	CTX ^R , CPM ^R , ATM ^R , IMI ^R , ETP ^R , TET ^R	29 (65.91)	0.55
3	4	CTX ^R , CPM ^R , ETP ^R , TET ^R	38 (86.36)	0.36
3	3	CPM ^R , ETP ^R , TET ^R	44 (100.0)	0.27

Legend: CTX: Cefotaxime, CPM: Cefepime, CTR: Ceftriaxone, ATM: Aztreonam, PIP: Piperacillin, IMI: Imipenem, ETP: Ertapenem, GEN: Gentamicin, KAN: Kanamycin, TET: Tetracycline, CIP: Ciprofloxacin.

DISCUSSION

The elucidation of disease-causing organisms from captive animals is significant for implementation of preventive, control and surveillance programs of diseases, as well as for implementing public and animal health policies (Jardine *et al.*, 2011). The incidence of salmonellosis and toxigenic *E. coli* in individuals despite public health education has remained unchanged in the last 2 decades, with a shift in serotype dissemination from foodborne-associated strains towards environmentally-distributed strains, from which the routes are not always recognized (CDC, 2014). The present study has determined the population count and antimicrobial resistance profile of *Escherichia coli* and *Salmonella* species detected from the faeces of some captive animals in Ogba zoo, Benin City, Nigeria. The infectious dose of *Salmonella* has been reported to be $> 10^5$ while that of *E. coli* have been described to be between 10^6 - 10^8 organisms respectively. However, the infection may also be established at lower densities (Hara-Kudo and Takatori, 2011). The presence of high densities of the *Salmonella* and *E. coli* observed in this study coupled with the antibiotic-resistant index of the isolates establishes captive animals as a potential reservoir of pathogenic and antibiotic-resistant *Salmonella* and *E. coli* in the environment. Both disease-causing agents have been detected from several species of captive and wildlife origin (Hassan and Shobrak, 2014; Suphoronski *et al.*, 2015; Hayashimoto *et al.*, 2016; Hernandez *et al.*, 2016). *Salmonella* has been elucidated from apparently healthy zoo animals (Stirling *et*

al., 2008), and has also been associated in disease outbreaks with significantly elevated mortality in humans (Chomel, 2007; Marchant *et al.*, 2016).

High level of prevalence of *Escherichia coli* and *Salmonella* species observed in the study, could be as a result of quality and storage of raw animal feeds, direct feeding by visitors, confinement conditions, contact with other captive animals and access of synanthropic wild animals (birds and rodents) (Conrad *et al.*, 2017). A study by Saleha *et al.* (2015) in Malaysia revealed that 27.5 - 61.0% snakes, 28.0% chelonians, 26.5 - 36.0% lizards and 31.0% house geckos harboured *Salmonella*. Hidalgo-Vila *et al.* (2007) reported an incidence of *Salmonellae* in land-dwelling turtles in Spain to be 100.0% compared to aquatic turtles (in streams and ponds,) which were between 12.0-15.4%. Boey *et al.* (2014) reported occurrence in captive lizards from zoological parks and pet shops in Malaysia to be 83.0% as likened to wild ones (in and around domiciliary areas) to be 25.0%. Lukac *et al.* (2015) revealed a study from apparently healthy reptiles, privately owned or at a zoo in Croatia to be 13.0% positive for *Salmonella*, 48.0% in lizards, 9.0% in snakes and 4.0% in turtles. Callaway *et al.* (2011) reported an occurrence of *Salmonella* in the intestinal subjects to be 7.0% from Asian house gecko in Australia. Chambers and Hulse (2006) reported that 95.3% of turtles and snakes investigated in the USA were *Salmonella* positive. These findings are somewhat similar to the findings of this study as all captive animals in this study harboured *Salmonella* species and *E. coli* in their faeces.

Literature have demonstrated a direct correlation between the prevalence of *Salmonella* shedding in captive animals and their proximity to anthropogenic activities/habitats (Une *et al.*, 2008; Daisuke, 2013). Urbanized animals have exclusive opportunities for exposure to enteric pathogen, infection, and dissemination resulting from various factors such as the quality of food and water they ingest, their comparatively sedentary lifestyles, the diversity of species with which they establish direct contact, as well as their consistent aggregations of elevated densities in small areas (Bradley and Altizer, 2007). Captive animals with a potential to be carriers of *Salmonella* and *E. coli* should be of significant interest due to their persistent contact with the public as well as their potential to contaminate environments inhabited by people.

With respect to the momentous temporal and spatial heterogeneity in antimicrobial resistance dissemination and the dynamics that upset its evolution, spread, and doggedness, it is imperative to pinpoint that antimicrobial resistance be seen as an ecological menace. Monitoring the prevalence of resistance of bacteria such as *Escherichia coli* and *Salmonella* in captive animals makes it conceivable to reveal that captive animals have the possibility to aid as a mixture and environmental reservoir of bacterial resistance. Hence it is important to address the issue of antimicrobial-resistant microorganism proliferating in the ecosystem and the environmental impact as well as related potential human health (Radhouani *et al.*, 2014).

The incidence of antimicrobial resistance in captive animals has numerous implications which include the prospect to serve as an ecological reservoir of bacterial resistance; the zoonotic prospect of enteric bacteria; and the potential drawbacks of the medical treatment of captive animals. The occurrence of antibiotic-resistant pathogenic *Salmonella* and *E. coli* is of public health principal concern globally predominantly in relation to the failure of the treatment regimen. Several literatures have showed an increase in the occurrence of antibiotic-resistant *Salmonella* and *E. coli* and the upsurge of multidrug-resistant strains in humans and animal populations with more than 80.0% isolates resistant to sulphonamides, tetracycline, chloramphenicol and streptomycin (Radhouani *et al.*, 2014; Saleha *et al.* 2015; Ones-Dias *et al.*, 2016). Reports of Abatcha *et al.* (2013) also showed resistance to cephalothin, cephalexin, and amoxicillin-clavulanic acid from captive animals. Fazhana *et al.* (2007) revealed that all isolates from zoo animals were resistant to erythromycin, while 50.0% of the isolates were resistant to ampicillin and

streptomycin. Nur-Mahiza *et al.* (2007) reported that 50.0% of the isolates were resistant to polymyxin B, followed by streptomycin and neomycin. Findings from these studies are similar to the findings in our present study. High multiple antibiotic resistant indexes observed in this study establishes the environments as a high-risk reservoir of antibiotic resistance. Hence, infections in humans emanating from such resistant salmonellae and *E. coli* in animals could be problematic and thus require stringent monitoring.

CONCLUSION

The present study has described the distribution and susceptibility profile of *Salmonella* species and *Escherichia coli* from captive animals in Ogba Zoological Garden. The presence of these potential pathogens coupled with their multidrug resistance potential call for public health concern. Further studies are required for the molecular characterization of the isolates to provide definitive evidence of resistance and virulence signatures coupled with zoonotic potential. The detection of these Enterobacteria suggests a significant risk of transmission between captive animals and close contacts, highlighting the necessity for staff training, improvement of hygienic procedures, public education and awareness of risks targeting visitors to chiefly utilize both educational and recreational activities of the catchment environment.

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