MIRJournal
Microbiology Independent Research Journal

DOI: 10.18527/2500-2236-2022-9-1-31-36

RESEARCH PAPER

Antibiotic-resistant salmonellae in pet reptiles in Saudi Arabia

Hajrah A. Khan[1,2], **Meshal H.K. Almalki**[1,2], **Rakan Felemban**[3], **Khaled Elbanna**[1,2,4], **Hussein H. Abulreesh**[1,2#]

[1]Department of Biology, Faculty of Applied Science, Umm Al-Qura University, Makkah, Saudi Arabia
[2]Research Laboratories Unit, Faculty of Applied Science, Umm Al-Qura University, Makkah, Saudi Arabia
[3]Alnoor Specialist Hospital, Ministry of Health, 3rd Ring Rd, An Naseem, Makkah, 2424, Saudi Arabia
[4]Fayoum University, Said Soliman St., University Region, Fayoum, 63514 Egypt

ABSTRACT

We investigated the occurrence rate of antibiotic-resistant salmonellae in exotic pet reptiles in Saudi Arabia. Salmonellae samples were collected from eight different genera of pet reptiles (snakes and lizards). Selective enrichment and selective plating procedures were carried out in order to detect salmonellae. Isolated bacteria were identified using biochemical tests, API 20E strips, and the VITEK compact system. Antimicrobial susceptibility testing was performed using the disc diffusion method. *Salmonella* spp. belonging to subspecies I (*Salmonella enterica* ssp. *enterica*) were detected in 29.2% of the samples. All of the detected salmonellae showed multidrug resistance ($p<0.001$, χ^2). The results demonstrated that pet reptiles in private households could present health hazards to humans. Therefore, these animals should be carefully handled to avoid infection. To the best of our knowledge, this is the first report regarding the occurrence rate of antibiotic-resistant salmonellae in pet reptiles in Saudi Arabia. The detected *Salmonella* serovars should be subjected to further in-depth molecular analyses in order to understand the overall epidemiology of salmonellosis in Saudi Arabia.

Keywords: pet reptiles, *Salmonella*, antimicrobial resistance

[#]**For correspondence**: Hussein H. Abulreesh, Vice Dean for Academic Affairs, Department of Biology, Faculty of Applied Science, Umm Al-Qura University, P. O. Box 7388, Makkah 21955, Saudi Arabia, e-mail: hhabulreesh@uqu.edu.sa

Citation: Khan HA, Almalki MHK, Felemban R, Elbanna Kh, Abulreesh HH. Antibiotic-resistant salmonellae in pet reptiles in Saudi Arabia. MIR J 2022; 9(1), 31–36. doi: 10.18527/2500-2236-2022-9-1-31-36.
Received: February 7, 2022
Accepted: February 24, 2022
Published: April 5, 2022

Conflict of interest: All of the authors declare no conflict of interest.
Funding: None
Acknowledgements: We are grateful to Mohammed Khalid Albar for allowing us to collect samples from the pet reptiles of a private household in Makkah.

INTRODUCTION

Human gastrointestinal infections that are caused by *Salmonella* species are of global concern. Salmonellae are ubiquitous in the environment, especially in the intestinal tract. *Salmonella enetrica* subsp. *enterica* (ssp. I) and subsp. *salame* (ssp. II) are commonly found in the intestinal tract of warm-blooded animals, whereas *Salmonella enterica* subsp. *arizonae* (ssp. IIIa), *diarizonae* (ssp. IIIb), *houtenae* (ssp. IV), and *indica* (ssp. VI) are known to inhabit the intestinal tract of cold-blooded animals, including amphibians and reptiles [1, 2]. The presence of Salmonellae in captive and free-living reptiles has been reported worldwide. *Salmonella* could infect reptiles with obvious clinical manifestations [3, 4]. Similarly, captive lizards, snakes, and turtles could also serve as asymptomatic reservoirs of these bacteria [5]. Salmonellae infections in pet reptiles could pose health risks to family members and pet handlers in zoos and pet shops. Adults of all ages, immunocompromised individuals, and children could contract salmonellosis either through direct contact with animals or with their fecal droppings [4, 5, 6, 7]. Reptile pets are becoming increasingly popular in Saudi Arabia but there is a lack of awareness regarding their link to salmonellosis. Therefore, the current study was conducted to assess the presence of salmonellae in pet reptiles in Saudi Arabia. Special attention was paid to the multidrug-resistant *Salmonella* strains and their potential health hazards to the animal handlers.

MATERIALS AND METHODS

Eight pet reptile species were obtained from a private household in Makkah, Saudi Arabia. The imported reptilian species included common boa (*Boa constrictor*) (n=1), Burmese python (*Python bivittatus*) (n=2), Schneider's skink (*Eumeces schneiderii*) (n=2), and mountain skink (*Plestiodon callicephalus*) (n=1). The hissing sand snake (*Psammophis sibilans*) (n=1) and the diadem snake (*Spalerosophis diadema*) (n=1) represented the local species. All of the reptiles belong to the same owner and were kept in separate cages with the exception of the two Schneider's skinks. Samples for the study were collected from the fecal droppings, cage swabs, and cloacal swabs of reptiles. All of the samples were collected in aseptic conditions. Three samples were collected for each reptile. In total, 24 samples were examined: eight fecal dropping samples, eight cloacal swab samples, and eight cage swab samples.

Salmonella was isolated by the pre-enrichment of samples at 37°C in buffered peptone water (BPW) (Oxoid, Basingstoke, UK) for 24 h. Positive BPW cultures were further subjected to selective enrichment at 41.5°C in Rappaport-Vassiliadis broth (Molecule-On, Auckland, New Zealand) for 24 h. All of the selective enrichment cultures were streaked on xylose lysine deoxycholate (XLD) (Oxoid) and CHROM agar Salmonella Plus plates (CHROMagar, Paris, France). Plates were aerobically incubated at 37°C for 24 h [8, 9].

The colonies that showed up as red with black centers on XLD agar and as mauve on CHROMagar Salmonella Plus were considered as presumptive salmonellae isolates. All of the presumptive salmonellae samples were subcultured on triple sugar iron agar (TSI) (Molecule-On) and Simmons' citrate slants (HiMedia, Mumbai, India) and incubated at 37°C for 24 h. The API 20E strips and VITEK 2 Compact system (bioMerieux, Marcy-I'Etoil, France) were used to confirm the salmonellae according to the manufacturer's instructions [9].

Then, the disc diffusion method was used for testing the antimicrobial susceptibility of all the confirmed *Salmonella* isolates according to the guidelines of the Clinical & Laboratory Standards Institute [10]. A 0.5 McFarland suspension of each isolate was spread on Mueller-Hinton agar (HiMedia) plates, incubated for 18 h at 37°C, and tested against antimicrobial agents that belong to nine different classes of antibiotics. Antimicrobial agents included ampicillin (10 µg), piperacillin (100 µg), ticarcillin (75 µg), amoxicillin/clavulanic acid (20/10 µg), piperacillin/tazobactam (30/6 µg) [penicillins], cefepime (30 µg), ceftazidime (30 µg) [cephalosporins], aztreonam (30 µg) [monobactams], imipenem (10 µg) [carbapenems], amikacin (30 µg), gentamicin (10 µg) [aminoglycosides], ciprofloxacin (1 µg) [fluoroquinolones], tetracycline (30 µg) [tetracyclines], chloramphenicol (30 µg) [phenicols], and nitrofurantoin (300 µg) [misc. agent]. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* NCTC 10662, and *Salmonella enterica* subspecies *enterica* serovar Typhimurium ATCC 14028 served as controls.

RESULTS

Salmonellae were detected in seven (29.2%) samples out of the total 24. For most of the pet reptiles, salmonellae were found in the fecal droppings. The diadem snake was an exception – in that case, bacteria were also found in the cloacal and cage swabs. The cage swabs of all other reptiles were negative for salmonellae (Table 1). A higher salmonellae presence in lizards was noted since salmonellae were detected in both lizard species (100%), whereas only 2 snake species (40%) tested positive for salmonellae (Table 1). All of the detected salmonellae belonged to *Salmonella enterica* ssp. *enterica* (subspecies I) and exhibited 100% antimicrobial resistance to ampicillin and ticarcillin (penicillins), cefepime and ceftazidime (cephalosporins), and aztreonam (monobactams) (Table 2). Two (8.3%) *Salmonella* isolates exhibited resistance to amoxicillin/clavulanic acid (penicillins), whereas five (20.8%) were resistant to gentamicin (aminoglycosides), ciprofloxacin (fluoroquinolones), tetracycline (tetracyclines), chloramphenicol (phenicols), and nitrofurantoin (misc. agent) (Table 2). However, none of the isolates demonstrated resistance against imipenem (carbapenems), piperacillin and piperacillin-tazobactam (penicillins), and amikacin (aminoglycosides).

Multidrug resistance (resistance to three or more antimicrobial classes) ($p<0.001$, χ^2) was observed in all of the isolated reptilian salmonellae in this study (100%, n=7). The resistance of salmonellae isolates to ampicillin, ticarcillin, cefepime, ceftazidime, and aztreonam was the most frequent resistance pattern (Table 3). One of the isolates exhibited a multidrug-resistance pattern to drugs that belong to five different antimicrobial classes, whereas two groups of three isolates demonstrated multidrug-resistance to drugs that belong to three and four antimicrobial classes (Table 3).

Table 1. Prevalence of *Salmonella* in pet reptiles

Reptile species	Origin	Sample type (number)	Number of positive samples	API ID	VITEK ID
Common boa	Imported	Feces (n=1)	1	*Salmonella* sp.	*Salmonella enterica* ssp. *enterica*
		Cloacal swab (n=1)	0	-	-
		Cage swab (n=1)	0	-	-
Burmese python	Imported	Feces (n=1)	0		-
		Cloacal swab (n=1)	0	-	-
		Cage swab (n=1)	0	-	-
Burmese python	Imported	Feces (n=1)	0	-	-
		Cloacal swab (n=1)	0	-	-
		Cage swab (n=1)	0	-	-
Hissing sand snake	Local	Feces (n=1)	0	-	-
		Cloacal swab (n=1)	0	-	-
		Cage swab (n=1)	0	-	-
Diadem snake	Local	Feces (n=1)	1	*Salmonella* sp.	*Salmonella enterica* ssp. *enterica*
		Cloacal swab (n=1)	1	*Salmonella* sp.	*Salmonella enterica* ssp. *enterica*
		Cage swab (n=1)	1	*Salmonella* sp.	*Salmonella enterica* ssp. *enterica*
Mountain skink	Imported	Feces (n=1)	1	*Salmonella* sp.	*Salmonella enterica* ssp. *enterica*
		Cloacal swab (n=1)	0	-	-
		Cage swab (n=1)	0	-	-
Schneider's skink	Imported	Feces (n=1)	1	*Salmonella* sp.	*Salmonella enterica* ssp. *enterica*
		Cloacal swab (n=1)	0	-	-
		Cage swab (n=1)	0	-	-
Schneider's skink	Imported	Feces (n=1)	1	*Salmonella* sp.	*Salmonella enterica* ssp. *enterica*
		Cloacal swabs (n=1)	0	-	-
		Cage swabs (n=1)	0	-	-
Total		24	7 (29.2%)		

Table 2. Antimicrobial susceptibility profiles of salmonellae isolated from pet reptiles

Salmonella isolates	Origin	Antimicrobial susceptibility profiles (zone diameter/mm)														
		AM	PRL	TC	AMC	TZP	CPM	CAZ	ATM	IMI	AK	GM	CIP	T	C	FT
A1	Common boa	R (0)	S (24)	R (0)	S (21)	S (25)	R (0)	R (0)	R (20)	S (30)	S (19)	S (19)	S (29)	S (18)	S (21)	R (10)
B1	Diadem snake	R (0)	S (21)	R (0)	R (12)	S (28)	R (0)	R (0)	R (19)	S (35)	S (20)	S (20)	S (25)	S (20)	S (20)	S (13)
B2	Diadem snake	R (0)	S (21)	R (0)	R (13)	S (28)	R (0)	R (0)	R (19)	S (35)	S (20)	S (19)	R (21)	S (20)	S (20)	S (13)
B3	Diadem snake	R (0)	S (21)	R (0)	S (16)	S (28)	R (0)	R (0)	R (19)	S (35)	S (20)	R (13)	S (25)	S (20)	S (20)	S (13)
C1	Mountain skink	R (0)	S (26)	R (0)	S (29)	S (30)	R (0)	R (0)	R (20)	S (38)	S (23)	S (21)	S (26)	R (13)	R (14)	S (19)
G1	Schneider's skink	R (0)	S (20)	R (0)	S (21)	S (34)	R (0)	R (0)	R (19)	S (32)	S (17)	S (19)	S (24)	S (16)	S (18)	S (11)
G2	Schneider's skink	R (0)	S (20)	R (0)	S (21)	S (34)	R (0)	R (0)	R (19)	S (32)	S (17)	S (19)	S (24)	S (16)	S (18)	S (11)
Number of resistant (total)		7	0	7	2	0	7	7	7	0	0	1	1	1	1	1

The resistance was determined by disc diffusion. S – sensitive, R – resistant, AM – ampicillin, PRL – piperacillin, TC – ticarcillin, AMC – amoxicillin-clavulanic acid, TZP – piperacillin-tazobactam, CPM – cefepime, CAZ – ceftazidime, ATM – aztreonam, IMI – imipenem, AK – amikacin, GM – gentamicin, CIP – ciprofloxacin, T – tetracycline, C – chloramphenicol, FT – nitrofurantoin

Table 3. Multidrug-resistance patterns of pet reptile-associated salmonellae

Salmonella isolates	Origin	Resistance pattern	Number of antimicrobial classes
A1	Common boa	AM, TC, CPM, CAZ, ATM, FT	4
B1	Diadem snake	AM, TC, AMC, CPM, CAZ, ATM,	3
B2	Diadem snake	AM, TC, AMC, CPM, CAZ, ATM, CIP	4
B3	Diadem snake	AM, TC, CPM, CAZ, ATM, GN	4
C1	Mountain skink	AM, TC, CPM, CAZ, ATM, T, C	5
G1	Schneider's skink	AM, TC, CPM, CAZ, ATM	3
G2	Schneider's skink	AM, TC, CPM, CAZ, ATM	3
P			$p<0.001$

AM – ampicillin, TC – ticarcillin, AMC – amoxicillin-clavulanic acid, CPM – cefepime, CAZ – ceftazidime, ATM – aztreonam, GM – gentamicin, CIP – ciprofloxacin, T – tetracycline, C – chloramphenicol, FT – nitrofurantoin.
The testing of the hypothesis that pet reptiles carry less abundant multidrug-resistant salmonellae isolates than non-multidrug-resistant ones was done by Pirson χ^2.

DISCUSSION

Wild and captive reptiles (turtles, snakes, and lizards) serve as symptomatic or asymptomatic carriers of exotic (subspecies *diarizonae* and *arizonae*) and human pathogenic (subspecies *enterica*) *Salmonella* serovars [2, 11, 12]. This study revealed the presence of *Salmonella* in two pet snakes (diadem snake and common boa) and two pet lizards (Schneider's skink and mountain skink) in a private household in Makkah, Saudi Arabia. Lukac et al. [11] have reported a higher salmonellae presence in lizards – 31 (48.4%) compared to 90 (8.9%) snakes. However, the relatively low number of samples examined, and the low number of isolates detected in the current study does not make it feasible to compare the prevalence of salmonellae in the reptiles reported in this study with other published results. The majority of the reptile-associated *Salmonella* serovars especially belonging to the subspecies *diarizonae, arizonae, houtenae,* and *salamae* are not pathogenic to humans [13]. However, human salmonellosis-associated serovars belonging to the subspecies (I) *Salmonella enterica* ssp. *enterica*, including Typhimurium, Entertidis, Paratyphi B, Kentucky, and Guinea, have been frequently reported in zoo and pet reptiles [2, 4, 11, 12, 14, 15]. All of the reptile salmonellae isolated in this study were identified as *Salmonella enterica* subsp. *enterica*. This subspecies poses potential hazards of containing human salmonellosis-associated serovars.

Multidrug resistance (MDR) – resistance to three or more antimicrobial classes – was noted in all of the bacteria isolated from the reptiles in this study. All of the *Salmonella* isolates showed resistance to ampicillin, ticarcillin, cefepime, ceftazidime, and aztreonam. Marin et al. [14] have reported multidrug resistance in 72% *Salmonella* species isolated from reptiles that are kept in private households and pet shops in Spain. Several studies have reported the reptile-associated *Salmonella* resistance to at least one antimicrobial agent [2, 14, 16, 17, 18]. Similarly, the results of the current study showed that resistance to ampicillin is common for the reptile-associated salmonellae [14, 16, 18]. The presence of the MDR reptile-salmonellae is associated with the animal diet [14]. A high abundance of salmonellae in the pet shop environment, which has been commonly reported, could easily lead to the infection of animals [14].

Contaminated animal feed can be a source of the *Salmonella* serovars in reptiles that can eventually lead to the salmonellosis of humans who take care of these animals [19]. The transfer of reptiles-associated salmonellosis via direct animal contact or fecal droppings have been reported in several studies [6, 13, 20]. Indirect reptile-associated salmonellosis transmission is also possible during the free-roaming of reptiles in the home, animal kissing, and cage cleaning [15]. In the UK, pet reptiles were reported to cause 27% of the *Salmonella* cases in children under the age of five [21]. *Salmonella* Guinea infections in patients younger than 5 years old in the US were found to be linked to the bearded dragon pet [15]. Approximately 7% of the salmonellosis cases in children in 16 US states (93,000 cases annually) in the period of 1996–1998 occurred through direct or indirect contact with pet reptiles (snakes, turtles, and lizards) [22]; that remains relevant today.

The pet reptiles containing multidrug-resistant *Salmonella* serovars that can lead to human salmonellosis are potentially hazardous in private households. The potential health risks related to improper reptile pet handling should be conveyed to the owners. The owners and pet handlers should also follow hygienic practices when handling pet reptiles. The areas in the house as well as the chairs and tables where the pet reptiles freely roam must be properly cleaned and disinfected. The feed of pet reptiles should also be kept away from the kitchen. To the best of our knowledge, this is the first study on the presence of antibiotic-resistant salmonellae in the pet reptiles of private households in Saudi Arabia. The identification of salmonellae serovars isolated during this study requires further molecular confirmation that will contribute to the further development of salmonellosis epidemiology in Saudi Arabia.

REFERENCES

1. Abulreesh HH. Salmonellae in the environment. Chapter 2. In: Annous BA. Gurtler JB. (eds), Salmonella: Distribution, Adaptation, Control Measures and Molecular Technologies. IntecOpen, Croatia, 2012; 19-50. doi: 10.5772/28201.
2. Zajac M, Skarzynska M, Lalak A, Kwit R, Smialowska-Weglinska A, Pasim P, et al. Salmonella in captive reptiles and their environment – can we tame the dragon. Microorganisms 2021; 9, 1012. doi: 10.3390/microorganisms901012.
3. Mitchell MA, Shane SM. Salmonella in reptiles. Semin Avian Exotic Pet Med 2001; 10, 25-35. doi: 10.1053/saep.2001.19798.
4. Bastos HM. Salmonella associated with snakes (suborder Serpentes). Chapter 3. In: Annous BA. Gurtler JB. (eds), Salmonella: Distribution, Adaptation, Control Measures and Molecular Technologies. IntecOpen, Croatia. 2012; 81-98. doi: 10.5772/30639.
5. Whiley H, Gardner MG, Ross K. A review of Salmonella and squamates (lizards, snakes and amphibians): implications for public health. Pathogens 2017; 6, 38. doi: 10.3390/pathogens6030038.
6. Kolker S, Itsekzon T, Yinnon AM, Lachish T. Osteomyelitis due to Salmonella enterica subsp. arizonae: the price of exotic pets. Clin Microbiol Infect 2012; 18, 167-70. doi: 10.1111/j.1469-0691.2011.03533. x.
7. Sauteur PMM, Relly C, Hug M, Wittenbrink MM, Berger C. Risk factors for invasive reptile-associated salmonellosis in children. Vector-Borne Zoonotic Dis 2013; 13, 419-21. doi: 10.1089/vbz.2012.1133.
8. Abulreesh HH, Paget TA, Goulder R. Waterfowl and the bacteriological quality of amenity ponds. J Water Health 2004; 2, 183-9. doi: 10.2166/wh.2004.0016.
9. Bjelland AM, Sandvik LM, Skarstein MM, Svendal L, Debenham JJ. Prevalence of Salmonella serovars isolated from retiles in Norwegian zoos. Acta Vet Scand 2020; 62, 3. doi: 10.1186/s13028-020-0502-0.
10. CLSI. Performance Standards for Antimicrobial Susceptibility Testing, 31st ed. CLSI supplement M100, March 2021. Clinical and Laboratory Standards Institute, USA.

11. Lukac M, Pedersen K, Prukner-Radovcic E. Prevalence of Salmonella in captive reptiles from Croatia. J Zoo Wildlife Med 2015; 46, 234-40. doi: 10.1638/2014-0098R1.1.
12. Tomastikova Z, Romero SB, Knotek Z, Karpiskova R. Prevalence and characteristics of Salmonella species isolated from captive reptiles in the Czech Republic. Vet Medicina 2017; 62, 456-69. doi: 10.17221/44/2017-VETMED.
13. Warwick C, Lambiris AJL, Westwood D. Reptile-related salmonellosis. J Royal Soc Med 2001; 94, 124-6. doi: 10.1177/014107680109400306.
14. Marin C, Lorenzo-Rebenaque L, Laso O, Villora-Gonzalez J, Vega S. Pet reptiles: a potential source of transmission of multidrug-resistant Salmonella. Front Vet Sci 2021; 7, 613718. doi: 10.3389/fvets.2020.613718.
15. Dewart CM, Waltenburg MA, Dietrich S, Machesky K, Singh A, Brandt E, de Fijter S. Cluster of human Salmonella Guinea infections: reported reptile exposures and associated opportunities for infection prevention – Ohio, 2019-2020. Prev Vet Med 2022; 198, 105530. doi: 10.1016/j.prevetmed.2021.105530.
16. Chen CY, Chen WC, Chin SC, Lai YH, Tung KC, Chiou CS, et al. Prevalence and antimicrobial susceptibility of salmonellae isolates from reptiles in Taiwan. J Vet Diag Invest 2010; 22, 44-50. doi: 10.1177/10406 3871002200107.
17. Bertelloni F, Chemaly M, Cerri D, le Gall F, Ebani VV. Salmonella infection in healthy pet reptiles: bacteriological isolation and study of some pathogenic characteristics. Acta Microbiol Immunolo Hungarica 2016; 63, 203-16. doi: 10.1556/030.63.2016.2.5.
18. McWhorter A, Owens J, Valcanis M, Olds L, Myers C, Smith I, et al. In vitro invasiveness and antimicrobial resistance of Salmonella enterica subspecies isolated from wild and captive reptiles. Zoonoses and Public Health 2021; 68, 204-12. doi: 10.111/zph.12820.
19. Goupil BA, Trent AM, Bender J, Olsen KE, Morningstar BR, Wunschmann A. A longitudinal study of Salmonella from snakes used in a public outreach program. J Zoo Wildlife Med 2012; 43, 836-41. doi: 10.1638/2011-0281R1.1.
20. Nagano N, Oana S, Nagano Y, Arakawa Y. A severe Salmonella enterica serotype Paratyphi B infection in a child related to a pet turtle, Trachemys scripta elegans. Jap J Infect Dis 2006; 59, 132-4. PMID: 16632917.
21. Mayor S. Over a quarter of Salmonella cases in English children are caused by pet reptile, a study finds. BMJ 2014; 349, G7796. doi: 101136/bmj. g7796.
22. JAMA. Reptile-associated salmonellosis-selected states, 1996-1998. JAMA 1999; 282, 2293-2294. doi: 10.1001/jama.282.24.2293.