*Review Article*

 Emerging Antibiotic Resistance in Salmonella: Implications for Wildlife

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ABSTRACT

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| Antibiotics play a crucial role in controlling bacterial infections; however, their overuse in human and veterinary medicine has led to the emergence of antibiotic resistance. This study examines antibiotic usage in veterinary medicine, the rise of antibiotic-resistant bacteria, and the role of reptiles in bacterial transmission. The widespread use of antibiotics in livestock, including prophylactic treatments, has contributed to resistance in bacterial pathogens such as *Salmonella*. Bacteria develop resistance through genetic mutations and horizontal gene transfer, making infections difficult to treat. Reptiles, including snakes and lizards, harbor diverse bacterial flora, including *Salmonella* spp., *Proteus* spp., *Pseudomonas* spp., and *Staphylococcus* spp., some of which are resistant to multiple antibiotics. Studies have shown that reptiles can act as reservoirs of zoonotic bacteria, contributing to the spread of antibiotic resistance. Research on *Salmonella* in captive and wild reptiles indicates a high prevalence of infection, particularly in carnivorous species. The increasing threat of antimicrobial resistance underscores the need for responsible antibiotic use, improved surveillance, and the development of new antimicrobial agents. Additionally, understanding the microbial diversity in wildlife, particularly reptiles, is essential to mitigate public health risks and prevent the spread of resistant pathogens |

*Keywords: Antibiotics, Bacteria, Resistance, Reptiles, Salmonella*

1. INTRODUCTION

Almost every continent and type of habitat is home to reptiles, one of the most diverse animal families. As a result of carrying and excreting Salmonella species in their feces, they frequently exhibit no symptoms. Salmonella infections have been linked to a variety of reptiles, including snakes, chameleons, iguanas, and turtles, in addition to the classical animals that are recognized to serve as reservoirs for *Salmonella* species [1, 2]). Several human Salmonellosis outbreaks linked to reptiles have been reported, and a great deal of research has been done to identify Salmonellae in both wild and captive reptiles (Kennedy, 1973). One of the known origins of human Salmonella infections is thought to be snakes. Snakes kept in captivity can contract Salmonella through their diet or by contact with wild snakes that are infected. Rodents that are either bred or imported from outside the facility are frequently used to feed captive reptiles (Goupil et al., 2012). The immune system is frequently weakened and bacteria are given a chance to colonize when the environment is not at its best and the lodging is unhygienic. Bacterial infections from the wild may be present in certain snakes, and once they are in captivity, clinical illnesses develop.

2. Antibiotics

Antibiotics are medications that kill or inhibit the growth of bacteria. Hence, they are used in the treatment of infectious bacterial diseases (Paterson *et al.*, 2016). In 1942 Waksman defined antibiotics as any substance produced by a microbe that inhibits or destroys the growth of other microorganisms (Woodruff, 2014). According to the article by Hamad (2010), only two new antibiotics were discovered during the past 30 years; oxazolidinone and linezolid which were discovered in 2000 and 2003 respectively.

**2.1 Antibiotic Usage in Veterinary Medicine**

Shortly after the development of antimicrobial drugs, they were used in veterinary medicine to treat mastitis in dairy cows. The antimicrobial drug usage varies between species and is dependent on several factors (Jhonston, 1998).Antimicrobial drugs were also used as growth promoters and as prophylactic agents (Silbergeld *et al.*, 2008) against respiratory diseases and liver abscesses (Doster *et al.*, 2018). Moreover, it was a common practice to treat the herds of livestock with antibiotics to avoid the spread of diseases which resulted in antibiotic overuse. In this scenario, uninfected animals were also exposed to antibiotics (Economou and Gousia, 2015). Streptomycin, gentamicin, kanamycin, penicillin, cefamandole and chloramphenicol are the most commonly used antibiotic drugs in veterinary practice.

**2.2 ANTIBIOTIC RESISTANCE**

Antibiotic resistance in bacteria was first reported by Rammelkamp and Maxon (1942). They reported that, before the discovery of penicillin there existed antibiotic resistant bacteria as scientists identified penicillinase as an enzyme in E. coli that inhibits the action of penicillin.Harrison and Lederberg (1998) defined antibiotic resistance as a defensive mechanism adopted by the microorganisms against the action of antibiotics.Bacteria became resistant to antimicrobial agents as a result of chromosomal changes or through the exchange of genetic material via plasmids and transposons. Tenovar (2006) explained that bacteria receive resistance genes by transformation, transduction, or conjugation. Large scale use of antibiotics in the community and hospitals has fuelled the crisis of antimicrobial resistance (Piddock, 2012). These pathogenic agents may be either resistant to single antibiotics or multiple antibiotics.

**2.2.1 Antibiotic Resistance in Salmonella**

Ejo *et al.* (2016) conducted a cross-sectoral study on the prevalence of antimicrobial resistant bacteria in the food items of animal origin in Brazil. The *Salmonella* isolates were resistant to tetracycline, amoxicillin, ampicillin, nitrofurantoin and cephalothin. The study concluded that the resistant *Salmonella* present in foods of animal origin can contribute to foodborne infections in Ethiopia.

**2.2.2 Emergence of Antibiotic- Resistant Bacteria**

The emergence of antibiotic resistance in different bacterial species has been a growing public health concern in recent decades. Antimicrobial resistance in bacterial pathogens is associated with high morbidity and mortality. The infections caused by resistant bacteria are difficult to treat and mostly untreatable with conventional antibiotics. Currently, there is a shortage of effective therapies, lack of adequate preventive measures, and overuse and abuse of antibiotics. Along with these, inappropriate drug prescription, large scale use in agriculture, as well as lack of new antibiotics contribute to the rise of antimicrobial resistance (Ventola, 2015; Frieri et al., 2017). The emergence of antibiotic resistance in bacteria affects the overall community and environmental health. This also led to an increase in the number of infections requiring costly treatment.

The resistant strains of bacteria are present in the environment and they circulate among humans, animals and the environment. According to a review by Rolain *et al.* (2012), antibiotic resistance involves interactions in an ecosystem among microbes, antibiotics, and resistant genes. The emergence of a new bacterial strain cannot be predicted. Antibiotic resistant genes naturally exist in the environment. Hence, studying different reservoirs and discovering new resistant genes in bacteria have to be carried out so that the emergence of multidrug resistance can be predicted. Also, this calls for the immediate need for developing new and more effective drugs that can combat the emerging crisis.

3. BACTERIAL DIVERSITY IN REPTILES

Goldstein *et al.* (1981) studied aerobic bacterial flora of Garter Snakes. An aggregate of 126 strains of aerobic and facultative bacteria was isolated from 82 Garter Snakes. Majority of these bacterial strains were potential human and snake pathogens. Among them, *Staphylococcus* spp. was the most common isolate. Three species of *Salmonella*, one *Shigella* isolate, and eight unidentified non- fermentative, gram-negative rods were also recovered. It was noted that it was quite possible that Garter Snakes could act as a reservoir of potential human pathogens. Soveri and Seuna (1986) examined samples taken from 23 captive non- venomous snakes for aerobic bacteria. The most prominent bacteria found were Gram-positive rods and Gram-positive cocci belonging to the family*Micrococcaceae*. *Salmonella virchow* was found in isolates from two snakes. They suggest that most of the bacterial flora isolated from the oral cavity may be occasional environmental bacteria.

Blaylock (2001) studied normal oral bacterial flora from Southern African snakes. In this study, eighteen snakes that belonged to 11 species were selected. Isolates obtained included members of *Enterobacteriaceae*, Gram-positive cocci, and anaerobes. Most of the bacterial isolates were, *Proteus* spp*.*, *Pseudomonas* spp*.*, *Salmonella arizonae* and *Staphylococcus epidermidis*.

A study conducted by Jho *et al.* (2011) identified bacterial flora in snakes imported from Vietnam. In this study, oral and cloacal samples were collected from eighteen Burmese Pythons imported from Vietnam. They isolated fourteen bacterial strains including *Aeromonas hydrophila*, *Citrobacter freundii*, *Corynebacterium jeikeium*, *Enterobacter spp.*, *Enterococcus spp.*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas spp.*, *Staphylococcus spp.* from both oral and cloacal samples.

A study by Lam *et al.* (2011) determined the patterns of oral bacterial flora and their sensitivity to antibiotics in captured native snakes in Hong Kong. Their result revealed that venomous snakes harboured more pathogenic bacteria. Among the venomous snakes, the Chinese Cobra (*Naja atra*) harboured the highest number of bacterial floras.

Dipineto *et al.* (2014) determined oral bacterial flora of *Python regius* kept as pets. The oral cavity examination of snakes revealed the presence of a wide range of gram-negative bacteria predominantly comprising of *Pseudomonas spp.*, *Morganella morganii*, *Acinetobacter calcoaceticus*, and *Aeromonas hydrophilia*. Additionally, *Salmonella spp.* was recorded in 15 per cent of tests and were serotyped as *S. blukwa*, *S. diarizonae*, *S. enteritidis* and *S. oranienburg*. The antimicrobial susceptibility testing showed that bacterial isolates were highly susceptible to enrofloxacin and ciprofloxacin.

Dehghani *et al.* (2016) identified bacterial flora in the oral cavity of snakes in Kahan, Iran. Samples were obtained from venomous and non-venomous snakes. The identified bacterial pathogens included species of the genera *Bacillus*, *Enterococcus*, *Escherichia*, *Proteus*, *Pseudomonas*, *Providencia*, *Salmonella* and *Staphylococcus*. This study revealed that both the venomous and non-venomous snakes harboured a wide variety of bacterial flora in their oral cavity. Hence, when these snakes bite a person, there is a risk of transferring these bacteria to the victims thereby increasing the bite complications. Therefore, along with antivenom treatment, the probability of causing bacterial infections also should be considered.

Artavia-León *et al.* (2017) determined the diversity of aerobic bacteria isolated from oral and cloacal cavities of free-living snakes in the Costa Rica rainforest. They examined both oral and cloacal swabs collected from the families *Viperidae* and *Colubridae*. Among the different bacteria identified, the most predominant bacterial species were *Aeromonas hydrophila*, *Bacillus* sp, *Enterococcus faecalis*, *Proteus hauseri*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Salmonella enterica diarizonae*, *Staphylococcus spp.* and *Stenotrophomonas maltophilia*. The study indicated that diverse bacteria found in snakes can act as opportunistic pathogens and enhance the risk of zoonosis in humans.

Shaikh *et al.* (2017) evaluated the oral microbiota of venomous snakes commonly found in India and assessed their antibiotic susceptibility. Oral swabs of 27 snakes representing Indian Cobra, Russell's Viper, Saw Scaled Viper, and Common Krait were selected for the study. The most common bacteria isolated from the samples were *Morganella morganii*, *Escherichia coli*, *Aeromonas aeruginosa*, *Staphylococcus aureus*, *Bacillus spp.*, *Micrococcus spp.* and *Clostridium perfringens*. The study showed that oral bacterial flora of snakes had great diversity of Gram-positive and Gram-negative bacteria.

Panda *et al.* (2018) examined the oral cavity of the Indian Cobra (*Naja naja*) for bacterial diversity. They collected oral swabs from six healthy Indian Cobras. Their microbiological examination indicated the dominance of Gram-negative bacteria over Gram-positive bacteria. The oral bacterial flora in Indian Cobra included bacteria such as *Salmonella* spp. (*S. typhi*, *S. paratyphi A*), *Pseudomonas* spp. (*P. aeruginosa*, *P. fluorescence*), *Proteus* spp. (*P. mirabilis*, *P. penneri*, *P. vulgaris*), *E. coli*, *Morganella* spp., *Citrobacter* spp. (*C. diversus*, *C. freundii*), *Aeromonas* spp. (*A. hydrophila*, *A. salmonicida*), *Enterobacter* spp. (*E. aerogens*), *Acinetobacter* spp. (*A. baumannii*), *Neisseria* spp., *Serratia* spp., *Bacillus* spp. (*B. cereus*, *B. megaterium*, *B. atrophaeus* and *B. weihenstephanensis*), *Enterococcus* spp. (*E. faecalis*, *E. faecium*), *Staphylococcus* spp. (*S. aureus*, *S. epidermidis*), *Alcaligenes* spp., *Chryseobacterium* spp. and *Micrococcus* spp. All the isolates were subjected to antimicrobial susceptibility testing. This study demonstrated that proper diagnosis and treatment should be provided for snake bite patients since oral bacteria might cause infections in the victims.

**4. SALMONELLOSIS IN ANIMALS**

The potential of reptiles as carriers of Salmonella and Arizona spp. was investigated by Iveson et al. (1969). The study indicated that there was no difference between the serotypes isolated from captive and wild reptiles. The study showed that lizards were more prone to Salmonella infections when compared to snakes. Kennedy (1973) isolated *Salmonella* spp. from snakes and other reptiles. Along with *Salmonella,* other bacteria including *Arizona hinshawii, Proteus* spp, *Citrobacter freundii*, and *Enterobacter* spp. were also isolated from the samples.

 Faldae *et al.* (1976) isolated *Salmonella* from captive animals in Nigeria. In this study they could isolate six serotypes of *Salmonella*, namely *Salmonella offa*, *Salmonella glostrup*, *Salmonella wimborne*, *Salmonella dublin*, *Salmonella saint paul* and *Salmonella webridge*. For the first time, *Salmonella wimborne* and*Salmonella glostrup* were reported in Nigeria. They also looked at the antibiotic susceptibility of all six serotypes. The result showed that all these strains were susceptible to nitrofurantoin and chloramphenicol but they were resistant to sulphafurazole and penicillin.

 Hoff *et al.* (1977) isolated *Salmonella* from free-ranging lizards in Florida. Ten isolates were positive for *Salmonella* and six *Salmonella* serotypes namely *S. flint*, *S. florida*, *S. gaminara*, *S. meunchen*, *S. miami* and *S. oranienburg* were recovered from these isolates.

Cambre *et al.* (1980) investigated the prevalence of *Salmonella* and *Arizona* in the reptiles housed at the National Zoological Park in Washington, DC. In this study, serotypes such as *Salmonella enteritidis*, *Salmonella choleraesuis* and *Arizona hinshawii* were recovered. This study also showed that snakes showed the highest rate of infection when compared to lizards and other reptiles.

Gugnani *et al.* (1986) isolated *Salmonella* and other enteropathogenic bacteria from Wall Geckos in Nigeria. They examined bacterial flora of 150 Wall Geckos (*Hemidactylus brookei*). A variety of bacteria including *Salmonella*, *Shigella sonnei*, *Edwardsiella tarda*, *Enterobacter* spp., *Citrobaeter freundii*, *Serratia marcescens*, *Proteus* spp., *Klebsiella pneumoniae* and *Escherichia coli* were isolated. The most prevalent bacteria were *Salmonella*. Among the eight serotypes of *Salmonella*, *S. hvittingfoss* and *S. typhimurium* were the most predominant serotypes.

Kalvig *et al.* (1991) isolated *Salmonella* from laboratory housed Iguanid lizards (*Sceloporus* spp). They captured lizards from the Costa Rica dry forest and housed them in the laboratory over a study period of 3 months. They isolated *Salmonella* spp. from the lizards. The study concluded that individual animals could develop systemic infections as a result of stress in captivity.

Burnham *et al.* (1998) conducted a cohort study on the prevalence of faecal shedding of *Salmonella* among captive Green Iguanas (*Iguana iguana*). All the 12Iguanas were positive for *Salmonella* and multiple serotypes were isolated from them.

Otokunefor *et al.* (2003) carried out a study to analyse the carriage rate of *Salmonella* in the gut and droppings of pest lizards in Nigeria. Three lizard species, namely the Agama Lizard (*Agama agama*), Wall Gecko (*Geckonidae*), and Snake Lizard (*Ameiva ameiva*) within the University of Port Harcourt campus and the University Village, were captured. Gut analysis showed a higher rate of *Salmonella* carriage in lizards that inhabited the outdoor environment namely the Agama and Snake Lizards than the Wall Geckos that dwelled indoors.

The prevalence of *Salmonella* in several reptiles was evaluated by Corrente *et al.* (2004). They isolated *Salmonella* strains and compared different culture media for isolation. Fifty percent of the samples were positive for *Salmonella* spp. and most of them belonged to *S. enterica*. The common serotypes recovered were *Salmonella newport*, *Salmonella muenster*, *Salmonella senftenberg*, *Salmonella aqua*, *Salmonella gaminara*, *Salmonella havana*, *Salmonella poona*, *Salmonella oldenburg* and *Salmonella minnesota*. The study confirmed the high prevalence of *Salmonella* strains in reptiles, especially in snakes and chameleons.

Ebani *et al.* (2005) investigated *Salmonella enterica* in the faeces of reptiles. In this study, *Salmonella* enterica was isolated particularly from Iguanas. A large number of *Salmonella* isolates were obtained from chelonians.

Palmgren *et al.* (2006) conducted a study on *Salmonella* in Black-headed Gulls (*Larus ridibundus*) in which they investigated the prevalence and *Salmonella* epidemiology. They found that 2.7 per cent of the individuals were positive for *Salmonella* and that the most predominating was *S. typhimurium*. All the *S. typhimurim* isolates were compared to the isolates from humans and domestic animals, and were found to be of the same serotype. The study showed that there existed a relationship between *S. typhimurium* isolates of gulls, humansand domestic animals indicating that Black-headed Gulls might play a role in the spread of *S. typhimurium.*

Scheelings *et al.* (2011) determined the prevalence of *Salmonella* in captive and wild reptiles. The result of this study revealed that *Salmonella* was more prevalent in captive reptiles. The most prevalent *Salmonella* serotype was *S. enterica diarizonae*. They suggested that the *Salmonella* prevalence was linked to diet. Carnivorous reptiles shed *Salmonella* more frequently in their faeces than insectivorous reptiles. Furthermore, captive reptiles that fed with rodents were positive for *Salmonella*.

Chiari *et al.* (2014) conducted a study in Northern Italy to isolate and identify *Salmonella* spp. from Red Foxes (*Vulpes vulpes*) and European Badgers (*Meles meles*). This study confirmed that the prevalence of *Salmonella* in foxes and badgers was low when compared to wild boars living in the same area. Different serovars of *S. enterica* were identified, among which some serovars were often associated with human illness. The result of the study demonstrated that opportunistic wild predators can indirectly infect both domestic animals and humans through the shedding of infectious pathogens into the environment.

Iovine *et al.* (2015) determined the presence of *S. enterica* and *E. coli* in free-ranging wild animals. They collected samples from two distinct regions in Brazil. The study showed that many wild animals harboured infectious pathogens and their presence might vary with respect to geographical locations.

Lukac *et al.* (2015) studied the prevalence of *Salmonella enterica* in captive reptiles. A total of 292 samples were collected from pet reptiles and the Zagreb Zoo, Croatia. The highest prevalence of *Salmonella* was observed in lizards followed by snakes and chelonians. *Salmonella enterica* serotypes such as, *Salmonella enterica arizonae*, *Salmonella enterica enterica* and *Salmonella enterica salamae* were identified. The study confirmed that the captive reptiles inCroatia harboured several serotypes of *Salmonella* which might act as reservoirs of reptile mediated Salmonellosis.

Mir *et al.* (2015) studied the occurrence and serotype diversity of *Salmonella* isolates in different species of poultry (chicken, emu, and duck) and determined their resistance pattern against various antibiotics of different classes. The serotyping results showed that the majority of isolates belonged to *Salmonella enteritidis*, followed by *Salmonella typhimurium*, *Salmonella virchow*, *Salmonella gallinarum*, *Salmonella reading*, and *Salmonella altona*. Their observations showed that poultry could serve as an important source of transmission of these antibiotic-resistant *Salmonella* serovars to humans.

Back *et al.* (2016) determined the prevalence of *Salmonella* spp. in pet turtles and their environment. They isolated *Salmonella* spp. from the faecal sample of 17 turtles. All these isolates were identified as *Salmonella enterica* through gene sequencing. This study showed that pet turtles distributed in Korea were infected with *Salmonella* spp. and their improper management would increase the risk of Salmonellosis.

Eugale *et al.* (2016) determined the prevalence and antimicrobial resistance of *Salmonella* in dairy cattle. They collected faecal samples from the peri-urban dairy farming facilities in Ethiopia where the interaction between animal and human population was very high. This study showed that the level of *Salmonella* prevalence was 2.3 per cent. Nine different *Salmonella* serotypes including *S. typhimurium*, *S. virchow* and *S. saintpaul* were identified.

Matias *et al.* (2016) isolated *Salmonella* from wild birds poached in illegal wildlife trade in Rio de Janeiro and investigated the prevalence of *Salmonella*. *Salmonella typhimurium* was isolated from Temminck’s Seed Eater (*Sporophila falcirostris*) and *Salmonella panama* was isolated from two Chestnut-capped Blackbirds (*Chrysomus ruficapillus*). These birds were kept in the same cage and had no symptoms of the disease. All the serovars showed multidrug resistance.They carried out pulse field gel electrophoresis (PFGE) analysis and the result showed 100 per cent similarity among the *Salmonella typhimurium* strain isolated from a Temminck’s Seed Eater and the strains isolated from a disease outbreak in humans, in Southern Brazil. The study indicated that trafficked wild animals could be a source of salmonellosis and that it could be responsible for disease outbreaks in animals and humans. The potential for dissemination of resistant *Salmonella* through wild birds and human sources might become a problem of public health concern.

Tomastikova *et al.* (2017) conducted a study in the Czech Republic in which they determined the prevalence and characteristics of the *Salmonella* strains from captive reptiles. They identified a total of 14 *Salmonella* serotypes. The most frequently found Serotypes were *S. enterica enterica*, *S. oranienburg*, *S. fluntern*, *S. tennessee* and *S. cotham*. The result of the study showed that the prevalence of *Salmonella* spp. was more in lizards followed by snakes and chelonians. *Salmonella* was more frequently detected in carnivorous or insectivorous reptiles than in omnivorous and herbivorous reptiles.

In a study, Silva *et al.* (2018) isolated *Salmonella* spp. in Cattle Egrets (*Bubulcus ibis*). The occurrence of *Salmonella newport* and *Salmonella typhimurium* suggested that Cattle Egrets may be reservoirs of different *Salmonella* serotypes and could cause a potential risk to public health and biological diversity.

A detailed review by Gutema *et al.* (2019) showed the diversity of *Salmonella* serotypes in healthy cattle across different countries. They concluded that the *Salmonella* serotypes such as *S. newport* and *S. typhimurium* were zoonotic pathogens.

Santos *et al.* (2020) investigated the presence of *Salmonella* spp. in wild birds from the Atlantic Forest in Brazil. Only one sample from *Ceratopipra rubrocapilla was positive for Salmonella enterica enterica. According to this study, a low prevalence of Salmonella spp. was observed in wild birds.*

**4.1 REPTILE RELATED SALMONELLOSIS**

As per a review by Warwick *et al.* (2001), *Salmonella* species commonly encountered in reptile mediated salmonellosis were *S. java*, *S. stanley*, *S. poona*, *S. mariana* and *S. panama*. The primary transmission route for reptile mediated salmonellosis was observed to be fecal-oral ingestion. However, terrapins, lizards and tortoises could transmit salmonellosis by claw scratches.

**5**. **SALMONELLA IN HUMAN-WILDLIFE INTERFACE**

In industrialized countries, non-typhoidal salmonellosis is considered as the most important foodborne infection and it can be considered as a public health problem. Humans can get salmonellosis either by direct contact (handling and exposure to infected animals) or by indirect contact via consumption of animal meat and food of animal origin or from contaminated food or water). Wild boar, wild birds and wild reptiles are the reservoirs of *Salmonella* and consumption of meat from these animals increases the incidence of salmonellosis in humans. Wild animals can act as a vector for transmitting the infections (Hilbert *et al.*, 2012).

4. Conclusion

Reptiles serve as reservoirs for antibiotic-resistant bacteria, posing a potential threat to public health. The misuse of antibiotics in veterinary care accelerates resistance, emphasizing the need for responsible antibiotic use, enhanced surveillance, and further research on microbial diversity in wildlife. Proactive measures can help mitigate the spread of resistant infections and safeguard both animal and human health.

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