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Aminoglycoside susceptibility and genetic characterization of *Salmonella enterica* subsp. *enterica* isolated from pet turtles

Sabrina Hossain, B.C.J. De Silva, S.H.M.P. Wimalasena, H.N.K.S. Pathirana, Gang-Joon Heo*

Veterinary Medical Center, College of Veterinary Medicine, Chungbuk National University, Cheongju 28644, Korea

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Abstract

Salmonella enterica subsp. *enterica* is a common microbial flora in pet turtles, which could opportunistically become pathogenic to human. Their possession of aminoglycoside resistance genes has important significance both in humans and animal medicine. In this study, twenty-one *Salmonella enterica* subsp. *enterica* were isolated from thirty-five individual turtles purchased from pet shops and online markets in Korea. In order to characterize the aminoglycoside susceptibility patterns, antimicrobial susceptibility tests were performed against gentamicin, amikacin and kanamycin of aminoglycoside antimicrobial group. Each of the isolates showed susceptibility to all tested aminoglycosides in disk diffusion and minimum inhibitory concentration (MIC) tests. PCR assay was carried out to determine aminoglycoside resistance genes, integron and integron mediated aminoglycoside genes. None of the isolates showed *aac*(*3*)-*IIa*, *aac*-(*6´*)-*Ib*, *armA*, *aphAI-IAB* aminoglycoside resistance genes. Only, five isolates (24%) harbored class 1 integron related *IntI1* integrase gene. The results suggest that *Salmonella enterica* subsp. *enterica* strains isolated from pet turtles are less resistance to aminoglycosides and don't harbor any aminoglycosides resistance genes.

Key words : *Salmonella enterica* subsp. *enterica*, Antimicrobial susceptibility tests, Aminoglycoside resistance genes, Class 1 integron, Pet turtles

INTRODUCTION

Salmonella is a genus of Gram-negative bacteria which causes numerous diseases in both humans and animals. It has been related to a broad spectrum of transferable diseases, comprising typhoid fever and non-typhoid salmonellosis which causes public health problems worldwide (Su and Chiu, 2007). The genus, *Salmonella* contains two species: *S. enterica* and *S. bongori*. *S. enterica* itself is divided into six subspecies such as *enterica*, salamae, arizonae, diarizonae, indica, and houtenae (Gomez et al, 1997). Among them, *Salmonella enterica* subsp. *enterica* is typically related to disease in warm-blooded animals and are usually diffused by ingestion of contaminated foods of animal and plant origins such as poultry, eggs, milk, beef, pork, fruits and vegetables or contaminated water with infected feces (Uzzau et al, 2000).

Aminoglycosides are used in the treatment of life-threatening bacterial infections in human and veterinary medicine. However, the extensive uses of aminoglycosides may result in aminoglycoside resistance. Aminoglycoside resistance is frequently associated with the presence of resistance genes and their related mechanisms. The most typical mechanism of aminoglycosides resistance is enzymatic modification, which is mediated by three classes of aminoglycoside-modifying enzymes such as acetyltransferases, nucleotidyltransferases and phosphotransferases (Ramirez and Tolmasky, 2010). In *Salmonella enterica* subspecies, *aac*(*3*)-*IIa* and *aphAI-IAB* genes encoding aminoglycoside acetyltransferase and phosphotransferase are frequently found (Miko et al, 2005; Lynne

*Corresponding author: Gang-Joon Heo, Tel. +82-43-261-2617, Fax. +82-43-267-3150, E-mail. gjheo@cbu.ac.kr

et al, 2008).

An alternative mechanism, methylation of the aminoacyl site of 16S rRNA, exhibits high-level resistance to medically significant aminoglycosides such as amikacin, tobramycin, and gentamicin (Doi and Arakawa, 2007). The methyltransferase gene, *armA* which is responsible for methylation of 16SrRNA was initially identified in the plasmid of clinical *Klebsiella pneumoniae* strain in 2003. Six types of 16S rRNA methyltransferase genes namely *armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD* and *npmA* conferring resistance to aminoglycosides, have been recognized so far (Hopkins et al, 2010). The *armA* gene is considered as the most common methyltransferase gene in *Salmonella enterica* as well as in other Enterobacteriaceae (Galimand et al, 2003).

Integrons are known as mobile genetic components that contribute to the dissemination of antimicrobial resistance determinants by horizontal or vertical transfer among the bacteria by a site-specific recombination mechanism involving a DNA integrase *intI*, recombination site *attl* and a strong promotor (Thungapathra et al, 2002). Three classes of integron such as class 1, 2 and 3 are the most studied and largely concerned in the spreading of antimicrobial resistance gene cassettes. Class 1 and 2 integrons are broadly distributed amongst all Gram-negative bacteria. In Salmonellae, Class 1 integron is most prevalent rather than Class 2 integron (Rodriguez et al, 2006).

Nowadays, turtles become popular as pet animals in worldwide. They are known as the reservoir of different pathogenic bacteria. Several bacterial species such as *Salmonella* spp., *Citrobacter freundii*, *Pseudomonas aeruginosa* were previously observed in healthy pet turtles (Back et al, 2016; Hossain et al, 2016; Wendt et al, 2017). Among them, *Salmonella* spp. was found to be most predominant bacteria. Even, turtles can harbor different strains of *Salmonella* without displaying any signs and symptoms of infection (Pfleger et al, 2003). In the 1970s, approximately 14% of human salmonellosis cases were described due to raising turtles (Lamm et al, 1972). Because of the emission of the zoonotic *Salmonella* spp. from the droppings of turtles, the sale of small carapace turtles (Carapace of <4 inches in length) was prohibited in 1975 in the United States' local pet shops by the U.S. Food and Drug Administration (FDA, 2016). Aminoglycosides especially gentamicin had been used to eradicate the *Salmonella* spp. and other bacteria from turtle eggs in some pet turtle farms in USA. Moreover, the use of such antimicrobials caused high-level resistance to aminoglycosides in *Salmonella enterica* subspecies that were isolated from pet turtles, turtle eggs and the pond water (Shane et al, 1990).

So far, turtle rearing has been continuously flourishing but it has not been corresponding to a suitable increase in therapeutic knowledge. The studies related to characterizing different aminoglycoside resistance genes of *Salmonella enterica* subsp. *enterica* isolates from pet turtles have not been conducted before. Therefore, the aim of the present study was to determine the aminoglycoside resistance patterns and to detect the aminoglycoside resistance genes in *Salmonella enterica* subsp. *enterica* isolated from six popular species of pet turtles in Korea and increase the awareness about the risk factors associated with the public health.

MATERIALS AND METHODS

Selection of pet turtles

Thirty-five of six commercially popular pet turtle species were bought from 9 pet shops and 8 online in several cities (Seoul, Daejeon and Cheongju) of Korea. The turtles were acquired with an average weight of 15±2 g, 40±5 mm of carapace diameter and under 4 weeks of age. Among 35 pet turtles, 11 Chinese stripe-necked turtles (*Ocadia sinensis*), 8 yellow bellied sliders (*Trachemys scripta scripta*), 6 river cooters (*Pseudemys concinna concinna*), 3 western painted turtles (*Chrysemys picta belli*), 3 common musk turtles (*Sternotherus odoratus*) and 4 northern Chinese softshell turtle (*Pelodiscus maackii*) were used for the study.

Bacterial isolation and identification

Fecal samples were taken from all of the turtles on the first day after purchasing. One mL of fecal sample was incubated in 9 mL of (GN) Gram-negative HAJNA broth (MBcell Ltd., Seoul, Korea) at 37°C for 24 h.

Then one loopful from each testtube was streaked onto a xylose lysine deoxycholate agar (MBcell Ltd, Seoul, Korea) and incubated at 37℃ for 24 h. Black colonies assumed for *Salmonella* spp. and were incubated onto a brilliant green agar (MBcell Ltd, Seoul, Korea) plate and incubated at 37℃ for 24 h. 16S rRNA sequencing was done with the universal primers 27F and 1492R in Cosmogenetech Co, Ltd. (Seoul, Korea) and sequencing analysis was performed by BLAST (National Center for Biotechnology Information, available through http://www.ncbi.nlm.nih.gov/BLAST/).

Antimicrobial susceptibility tests

Antimicrobial disk diffusion test was perceived with gentamicin, amikacin and kanamycin of aminoglycoside antimicrobial group on Mueller-Hinton agar (MBcell Ltd., Seoul, Korea). All the isolates were cultivated on tryptic soy agar (MBcell Ltd., Seoul, Korea) and generated colonies were matched with the turbidity of McFarland 0.5 (5×10^5 CFU/mL) standard by adding sterile saline. After incubating at 37℃ for 24 h, the zone of inhibition of isolates against tested aminoglycosides was measured. Minimum inhibitory concentrations (MIC) of gentamicin (0.03～16 μg/mL), amikacin (0.06～32 μg/mL) and kanamycin (0.06～32 μg/mL) were determined by broth microdilution method in a 96-well microtiter plate (Wiegand et al, 2008). Antimicrobial susceptibility tests were conducted according to the standards of Performance Standards for Antimicrobial Susceptibility Testing; Clinical and Laboratory Standards Institute (CLSI, 2014).

Detection of aminoglycoside resistance genes

PCR was carried out to detect aminoglycoside resistance genes such as *aac(3)-IIa*, *aac(6')-Ib*, *armA*, *aphAI-IAB* and *IntI1* by the selected primers (Table 1). The details of primers and conditions were obtained from Samadi et al. (2015), Diaz et al. (2006), Chenia (2016) and Frana et al. (2001). The PCR mixture, 25 μL contained 12 μL Quick Taq HS DyeMix (Toyobo Co., Ltd., Japan), 9 μL PCR water, 1 μL DNA template and 1 μL of each primer pairs. The thermal cycle for amplification of *aac(3)-IIa* gene consisted of 5 min initial denaturation at 94℃, 35 repeated cycles of 45 s at 94℃, 45 s at 55℃, 1 min at 72℃ and final extension at 72℃ for 10 min. The *armA* gene was amplified using conditions; 94℃ for 5 min, 35 cycles of 95℃ for 45 s, 53℃ for 30 s, 72℃ for 1 min and 72℃ for 10 min. For amplification of *aphA-IAB* gene comprised of 5 min initial denaturation at 94℃, 40 repeated cycles of 1 min at 94℃, 30 s at 53℃, 30 s at 72℃ and final extension at 72℃ for 7 min PCR conditions of *aac(6')-Ib* were 94℃ for 45 s, 55℃ for 45 s, 72℃ for 45 s and 72℃ for 10 min with 34 cycles. The PCR products were observed in 1.5% (W/V) agarose gel with gel loading buffer and DNA stain (Jena Bioscience GmbH, Germany).

Table 1. Nucleotide Sequence of Oligonucleotide Primers Used in the study

Targeted gene	Primers	Nucleotide sequences (5'-3')	Size of fragment (bp)	References
aac(3)-IIa	F	ATGGGCATC ATTCGCACA	749	Samadi et al. 2015
	R	TCTCGGCTTGAACGAATTGT		
armA	F	AGGTTGTTTCCATTTCTGAG	591	Samadi et al. 2015
	R	TCTCTTCCATTCCCTTCTCC		
IntI1	F	CTACCT CTCACTAGTGAGGGGCGG	845	Diaz et al. 2006
	R	GGG CAGCAGCGAAGTCGAGGC		
apha1-IAB	F	AAACGTCTTGCTCGA GGC	500	Frana et al. 2001
	R	CAAACCGTTATTCATTCGTGA		
aac(6')-Ib	F	TTGCGATGCTCTATGAGTGGCTA	482	Chenia 2016
	R	CTCGAATGCCTGGCGTGTTT		

RESULTS

Identification of *Salmonella enterica* subsp. *enterica*

Twenty-one *Salmonella enterica* subsp. *enterica* confirmed by 16S rRNA gene sequencing were isolated from turtles as follows; 9 Chinese stripe-necked turtles, 4 yellow bellied sliders, 3 river cooters, 1 western painted turtles, 2 common musk turtles and 2 northern Chinese softshell turtles.

Aminoglycosides resistance patterns

The disk diffusion and MIC results are shown in Table 2. In disk diffusion test, each of the isolates was susceptible against tested aminoglycosides. All of the isolates showed low MIC values in MIC test. The MIC values of the isolates were acquired from gentamicin (2～4 μg/mL), amikacin (2～8 μg/mL) and kanamycin (4～8 μg/mL).

Detection of aminoglycoside resistance genes and class 1 integron gene

According to the PCR results, none of the isolates contained *aac(3)-IIa*, *aac(6´)-Ib*, *armA*, *aphAI-IAB* aminoglycoside resistance genes. However, Only five isolates (24%) from 2 Chinese stripe necked turtle, 1 river cooter, 1 yellow bellied turtle and 1 northern softshell turtle harbored class 1 integron-integrase *Int1* gene.

DISCUSSION

Zoonotic *Salmonella enterica* subsp. *enterica* has been found to cause salmonellosis in animals and humans. It has also been observed to grow antimicrobial resistance

Table 2. Aminoglycoside resistance patterns of *Salmonella enterica* subsp. *enterica* isolated from pet turtles

Isolate*	Disk diffusion zone diameters (mm)**			MIC (μg/mL)**		
	GEN10	AK30	K30	GEN	AK	K
CSN1	24(S)	31(S)	30(S)	4(S)	2(S)	8(S)
CSN2	19(S)	25(S)	24(S)	2(S)	2(S)	8(S)
CSN3	23(S)	20(S)	24(S)	2(S)	4(S)	4(S)
CSN4	22(S)	24(S)	22(S)	4(S)	2(S)	8(S)
CSN5	21(S)	25(S)	23(S)	4(S)	8(S)	8(S)
CSN6	19(S)	24(S)	23(S)	2(S)	4(S)	8(S)
CSN7	20(S)	22(S)	21(S)	4(S)	4(S)	8(S)
CSN8	20(S)	22(S)	21(S)	4(S)	4(S)	8(S)
CSN9	18(S)	21(S)	22(S)	2(S)	8(S)	8(S)
YB1	21(S)	24(S)	24(S)	4(S)	4(S)	8(S)
YB2	26(S)	26(S)	25(S)	4(S)	4(S)	8(S)
YB3	20(S)	22(S)	22(S)	4(S)	2(S)	8(S)
YB4	20(S)	24(S)	22(S)	2(S)	2(S)	8(S)
RC1	22(S)	23(S)	22(S)	4(S)	4(S)	8(S)
RC2	20(S)	24(S)	22(S)	4(S)	4(S)	8(S)
RC3	18(S)	24(S)	22(S)	4(S)	2(S)	8(S)
CM1	20(S)	23(S)	22(S)	4(S)	4(S)	8(S)
CM2	18(S)	22(S)	21(S)	4(S)	8(S)	16(S)
CSS1	20(S)	25(S)	24(S)	4(S)	4(S)	8(S)
CSS2	22(S)	26(S)	24(S)	4(S)	8(S)	4(S)
WP	22(S)	24(S)	27(S)	4(S)	2(S)	8(S)

**Salmonella enterica* subsp. *enterica* from Chinese stripe-necked turtle, yellow bellied slider, river cooter, common musk, northern Chinese softshell turtle and western painted turtle were marked as CSN, YB, RC, CM, CSS and WP, respectively.

** Disk diffusion zone diameters (mm) and MIC (μg/mL) values were described following the standards of Clinical Laboratory Standards Institute (CLSI 2014); Where, S: Susceptible.

especially aminoglycoside resistance. Various resistance mechanisms and determinants have been previously studied which was alarmed to public health. Therefore, the research of antimicrobial resistance concerning aminoglycoside resistance of *Salmonella enterica* subsp. *enterica* has important significance.

The present study was conducted to characterize aminoglycoside susceptibility and aminoglycoside resistance genes of *Salmonella enterica* subsp. *enterica* isolates from pet turtles. In accordance with antimicrobial susceptibility testing results, all strains were susceptible to three aminoglycosides such as gentamicin, amikacin and kanamycin. According to a recent study, *Salmonella enterica* subspecies from healthy pet reptiles were reported to be susceptible to gentamicin, amikacin, kanamycin and tobramycin (Bertelloni et al, 2016). In another study, *Salmonella enterica* subsp. *enterica* isolates from Russian tortoises showed susceptibility against gentamicin (Nowakiewicz et al, 2012). Comparatively similar results were found with 95% susceptibility to gentamicin from *Salmonella* serovars which were isolated from domestic turtles (Chen et al, 2010). On the contrary, human clinical strains of *Salmonella enterica* displayed high resistance to clinically used aminoglycosides. Even, aminoglycoside resistant *Salmonella enterica* subspecies were found in chicken, swine, turkey and cattle (Lynne et al, 2008).

In this study, only five isolates (24%) contained Class 1 Integron-integrase gene *IntI*. The existence of class 1 integron is more or less related to the multiresistance characteristics of *Salmonella enterica* isolates (Naghoni et al, 2010). In our case, the isolates which harbored *Int1* gene were susceptible to all tested aminoglycosides. Previously, several gene cassetes were obtained from *Int1* positive isolates which were resistant to different antimicrobials indicating the presence of *Int1* gene was not only limited to aminoglycoside resistance (Thungapathra et al, 2002). Although, the *aac*(*6´*)-*Ib* gene was most frequently found as a gene cassette of class 1 integron, the current study failed to detect the gene (Ramirez et al, 2010). As any integron mediated aminoglycoside resistance genes were not found, the study could not relate the incidence of class 1 integron gene with aminoglycoside resistance genes. The occurrence of class 1 integron mediated genes may be resulted due to the other antimicrobial resistance genes of different antimicrobial group. Since, there are several antimicrobial resistance genes were previously found as gene cassettes of class 1 integrons in *Salmonella enterica* strains which are resistant to other antimicrobial groups (Rodrıguez et al, 2006).

With regards to aminoglycoside resistance genes characterization, none of the isolates harbored *armA*, *aphAI-IAB*, *aac*(*3*)-*IIa* genes. Lynne et al. (2009) and Hopkins et al. (2010) have detected *armA* and *aphA-IAB* genes in highly amikacin and kanamycin resistant *Salmonella enterica* isolates from human and domestic animals. According to a recent study, the *aac*(*3*)-*IIa* gene was detected in *Salmonella enterica* subsp. *enterica* isolates from pig farm conferring a high level of aminoglycoside resistance (Lopes et al, 2015). However, the presence of the aminoglycoside acetyltransferase gene, *aac*(*3*)-*IIa* has not always been reported to exhibit aminoglycoside resistance patterns (Vakulenko et al, 2003). The absence of *aac*(*3*)-*IIa* gene was reported in kanamycin resistant *Salmonella* strains in hospital (Samadi et al, 2015).

According to the personal communication with pet shops, the turtles which were purchased and used in this study were very young. All of the turtles were healthy and no sign of diseases was observed. Even, Aminoglycosides was not exposed after turtle hatching. A study was investigated about *Salmonella enterica* subsp. *enterica* strains isolated from gentamicin treated and untreated turtle eggs in a pet turtle farms. In the study, gentamicin treated isolates showed high aminoglycoside resistance and untreated isolates showed gentamicin susceptibility (Diaz et al, 2006). As no antimicrobial was used, it could be the reason of *Salmonella enterica* subsp. *enterica* to be more susceptible to all aminoglycosides and consuming no aminoglycosides resistance genes.

It is reasonable that the *Salmonella enterica* subsp. *enterica* isolated from six different turtle species are susceptible to all aminoglycosides and lacking in aminoglycoside resistance genes. So, it can be a positive fact for the people who keep turtles as a pet. The reason behind the presence of integron in the isolates could not

figure out in the current study. Therefore, the further research should be focused on the studies more related to different classes of integron and integron mediated antimicrobial resistance gene cassettes in *Salmonella enterica* subsp. *enterica*.

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CONFLICT OF INTERESTS

There is no conflict of interests declared.

REFERENCES

Back DS, Shin GW, Wendt M, Heo GJ. 2016. Prevalence of *Salmonella* spp. in pet turtles and their environment. Lab Anim Res. 32: 166-170. doi: 10.5625/lar.2016.32.3.166.

Bertelloni F, Chemaly M, Cerri D, Gall FL, Ebani VV. 2016. *Salmonella* infection in healthy pet reptiles: Bacteriological isolation and study of some pathogenic characters. Acta Microbiol Immunol Hung. 63: 203-216.

Chen TH, Lue KY. 2010. Population status and distribution of freshwater turtles in Taiwan. Oryx. 44: 261-266.

Chenia HY. 2016. Prevalence and characterization of plasmid-mediated quinolone resistance genes in *Aeromonas* spp. isolated from South African freshwater fish. Int J Food Microbiol. 231: 26-32.

CLSI. 2014. Performance standards for antimicrobial susceptibility testing: Twenty-fourth informational supplement. CLSI M100-S24, Clinical and Laboratory Standards Institute (CLSI), Wayne, PA., USA.

Diaz, MA, Cooper RK, Cloeckaert A, Siebeling RJ. 2006. Plasmid-mediated high-level gentamicin resistance among enteric bacteria isolated from pet turtles in Louisiana. Appl Environ Microbiol. 72: 306-312.

FDA. 2016. Pet turtles: Cute but commonly contaminated with *Salmonella*. http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm048151.htm.

Frana TS, Carlson SA, Griffith RW. 2001. Relative distribution and conservation of genes encoding aminoglycoside-modifying enzymes in *Salmonella enterica* serotype Typhimurium phage type DT104. Appl Environ Microbiol. 67: 445-448.

Galimand M, Courvalin P, Lambert T. 2003. Plasmid-mediated high-Level resistance to aminoglycosides in Enterobacteriaceae due to 16S rRNA methylation. 2003. Antimicrob Agents Chemother. 47: 2565-2571. doi:10.1128/AAC.47.8.2565-2571.2003.

Gomez TM, Motarjemi Y, Miyagawa S, Kaferstein FK, Stohr K. 1997. Foodborne salmonellosis. World Health Stat Q. 50: 81-89.

Hopkins KL, Escudero JA, Hidalgo L, Gonzalez-Zorn B. 2010. 16S rRNA methyltransferase RmtC in *Salmonella enterica* Serovar Virchow. Emerg Infect Dis. 16: 712-715. doi:10.3201/eid1604.090736.

Hossain S, Wimalasena SHMP, Heo GJ. 2017. Virulence factors and antimicrobial resistance pattern of *Citrobacter freundii* isolated from healthy pet turtles and their environment. Asian J Anim Vet Adv. 12: 10-16.

Lamm SH, Taylor A Jr, Gangarosa EJ, Anderson HW, Young W, Clark MH, Bruce AR. 1972. Turtle-associated salmonellosis. I. an estimation of the magnitude of the problem in the United States, 1970-1971. Am J Epidemiol. 95: 511-517.

Lopes GV, Pissetti C, da Cruz Payao Pellegrini D, da Silva LE, Cardoso M. 2015. Resistance phenotypes and genotypes of Salmonella enterica subsp. enterica isolates from feed, pigs, caracasses in Brazil. J Food Prot. 78: 407-413.

Lynne AM, Rhodes-Clark BS, Bliven K, Zhao S, Foley, SL. 2008. Antimicrobial Resistance genes associated with *Salmonella enterica* Serovar Newport Isolates from food animals. Antimicrob Agents Chemother. 52: 353-356.

Miko A, Pries K, Schroeter A, Helmuth R. 2005. Molecular mechanisms of resistance in multidrug-resistant serovars of *Salmonella enterica* isolated from foods in Germany. J Antimicrob Chemother. 56: 1025-1033.

Naghoni A, Ranjbar R, Tabaraie B, Farshad S, Owlia P, Safiri Z, Mammina C. 2010. High prevalence of integron mediated resistance in clinical isolates of *Salmonella enterica*. Jpn J Infect Dis. 63: 417-421.

Nowakiewicz A, Ziolkowska G, Zieba P, Stepniewska K, Tokarzewski S. 2012. Russian tortoises (*Agrionemys horsfieldi*) as a potential reservoir for *Salmonella* spp. Res Vet Sci. 92: 187-190.

Pfleger S, Benyr G, Sommer R, Hassl A. 2003. Pattern of *Salmonella* excretion in amphibians and reptiles in a vivarium. Int J Hyg Environ Health 206: 53-59.

Ploy MC, Lambert T, Couty JP. 2000. Integrons: an antibiotic resistance gene capture and expression system. Clin. Chem Lab Med. 38: 483-487.

Ramirez MS, Tolmasky ME. 2010. Aminoglycoside modifying enzymes. Drug Resist Updat 13: 151-171.

Rodriguez I, Rodicio MR, Mendoza MC, Cruz Martín M. 2006. Large conjugative plasmids from clinical strains of *Salmonella enterica* serovar Virchow contain a class 2 integron in addition to class 1 integrons and several non-integron-associated drug resistance determinants. Antimicrob Agents Chemother. 50: 1603-1607.

Samadi N, Pakzad I, Monadi Sefidan A, Hosainzadegan H, Tanomand A. 2015. study of aminoglycoside resistance genes in *Enterococcus* and *Salmonella* strains isolated from Ilam and Milad hospitals, Iran. *Jundishapur Journal of Microbiology*. 8: e18102. doi:10.5812/jjm.8(4)2015.18102.

Shane SM, Gilbert R, Harrington KS. 1990. *Salmonella* colonization in commercial pet turtles (*Pseudemys scripta elegans*). Epidemiol Infect. 105: 307-16.

Su LH, Chiu CH. 2007. *Salmonella*: Clinical importance and evolution of nomenclature. Chang Gung Med. J. 30: 210-9.

Thungapathra M, Amita, Sinha KK, Chaudhuri SR, Garg P, Ramamurthy T, Nair GB, Ghosh A. 2002. Occurrence of antibiotic resistance gene cassettes *aac(6′)-Ib*, *dfrA5*, *dfA12*, and *ereA2* in Class I Integrons in Non-O1, Non-O139 *Vibrio cholerae* strains in India. 46: 2948-2955.

Uzzau S, Brown DJ, Wallis T, Rubino S, Leori G, Bernard S, Casadesus J, Platt DJ, Olsen JE. 2000. Host adapted serotypes of *Salmonella enterica*. Epidemiol Infect. 125: 229-255.

Vakulenko SB, Mobashery S. 2003. Versatility of aminoglycosides and prospects for their future. Clin Microbiol Rev. 16: 430-450.

Wendt M, Heo GJ. 2016. Multilocus sequence typing analysis of *Pseudomonas aeruginosa* isolated from pet Chinese stripe-necked turtles (*Ocadia sinensis*). Lab Anim Res. 32: 208-216.

Wiegand I, Hilpert K, Hancock RE. 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Prot. 3: 163-175.