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***Salmonella* serovars and antimicrobial resistance in strains isolated from wild animals in captivity in Sinaloa, Mexico**

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Abstract

The aim of the present study was to evaluate the frequency of antibiotic resistance in *Salmonella* spp. strains from wild animals in captivity at the Culiacan Zoo and the Mazatlan Aquarium in Sinaloa, Mexico. We identified 17 different *Salmonella enterica* serovars at a prevalence of 19.90% (Culiacan Zoo) and 6.25% (Mazatlan Aquarium). Antibiotic sensitivity tests revealed that, of the 83 strains studied, 100% were multidrug resistant (MDR). The drugs against which the greatest resistance was observed were: penicillin, erythromycin, dicloxacillin, ampicillin, cephalothin, and chloramphenicol. We therefore conclude that MDR is common among *Salmonella* isolates originating from wild animals in captivity in Sinaloa.

Key Words: antibiotic resistance, aquarium, zoo

Ninety-nine percent of salmonellosis in humans and animals is attributed to serovars of the *enterica* species [15]. Intestinal salmonellosis, which is primarily associated with *enterica* species and foodborne toxi-infections, is generally resolved within five to seven days; however, some cases may progress to bacteraemia, requiring

antibiotic therapy [8]. In Mexico, a government study conducted in 2008 reported that three million deaths are caused by intestinal infections such as Salmonellosis due to consumption of contaminated water or food, and Sinaloa is one of the states with the highest number of salmonellosis cases in the country [6].

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Little is known regarding the role captive wild animals play as reservoirs of *Salmonella*; however, it is known that the number of animals affected by this genus has increased and that these isolates show marked antibiotic resistance [7]. Two examples of this phenomenon are the Valle del Emperador Zoo in Trinidad and the Seoul Grand Park in Korea where the prevalence of *Salmonella* excretion was 7% (99% with antibacterial resistance) and 5.8% (47% with antibacterial resistance), respectively [9,14]. In addition to antimicrobial resistance, the administration of antibiotics reduces intestinal microbiota, which is associated with the long-term injurious impact on resistance to *Salmonella* colonisation in the intestine, possibly leading to greater faecal excretion of the pathogen and the carriage state [2].

The aim of this study was to evaluate the frequency of antibiotic resistance in *Salmonella* spp. strains isolated from captive wild animals at the Culiacan Zoo and Mazatlan Aquarium in Sinaloa, Mexico.

A prospective study was carried out between November 2010 and June 2011. Faeces, water, and food samples were collected from each cage at the Culiacan Zoo and the Mazatlan Aquarium, as well as from biological filters from the latter. In total, 636 samples were collected: 316 from the zoo and 320 from the aquarium, which were placed into sterile wide-mouth flasks identified with the corresponding shelter number, type of food, or biological filter. The samples were taken transported to the Bacteriology Laboratory, Faculty of Veterinary Medicine and Animal Husbandry at the Autonomous University of Sinaloa. Isolation was then performed the same day followed by inoculation in tetrathionate broth as an initial pre-enrichment medium, Rappaport-Vassiliadis as selective enrichment and XLT4 agar as a selective medium, according to the method of Love and Rostagno [11]. The bacterial colonies displaying a typical *Salmonella* genus morphology on the XLT4 agar base (black coloration of the colonies) were submitted for confirmatory

biochemical analysis. The biochemical media and tests performed were: sorbitol broth, mucate broth, motility-indole-ornithine medium (MIO), triple sugar iron (TSI), lysine iron agar (LIA), and citrate and urease tests. Serotyping was performed according to the Kauffman-White scheme by the Institute of Epidemiological Diagnosis and Reference (InDRE). Testing for antibiotic resistance was performed in triplicate using the disc diffusion method as described by the National Committee for Clinical Laboratory Standard (NCCLS), for amikacin (AK-30 µg), ampicillin (AM-10 µg), cephalothin (CP-30 µg), ceftriaxone (CRO-30 µg), chloramphenicol (CL-30 µg), dicloxacillin (DC-1 µg), enoxacin (ENX-10 µg), erythromycin (E-15 µg), gentamicin (GE-10 µg), netilmicin (NET-30 µg), penicillin (PE-10 U) and trimethoprim- sulfamethoxazole (SXT-25 µg). *S. Typhimurium* ATCC 14028S was used for quality control. The isolates that showed resistance to three or more antibiotics belonging to different families were considered multidrug-resistant (MDR).

Of the samples collected at the zoo, 63 isolates were obtained representing a total prevalence of 19.9% (95% confidence interval [CI], 15.3-24.5). At the aquarium, 20 isolates representing a total prevalence of 6.25% (95% CI, 3.442-9.058) were obtained. Seventeen serovars apart from *S. enterica* were identified, from which the most frequently isolated at the zoo were *S. Albany* (44%), *S. (3,10, H: r:-)* (11%) and *S. San Diego* (9%), while the most frequently isolated at the aquarium were *S. Poona* (25%), *S. Saintpaul* (20%), and *S. Panama* (10%) (Table 1).

Antibiotic sensitivity tests found that all 83 strains studied were resistant to at least three of the 12 drugs tested. The drugs against which the greatest resistance was shown were: penicillin, erythromycin, and dicloxacillin (83 strains or 100%), followed by ampicillin (62 strains or 74.6%), cephalothin (8 strains or 9.6%), and chloramphenicol (6 strains or 7.2%) (Table 2). Four different resistance patterns, as well as MDR in 100% of strains analysed, were identified.

Table 1. *Salmonella enterica* serovars identified at the Culiacan Zoo and Mazatlan Aquarium

Recreation park	Serovars	Positive cages or enclosures (# of isolates)	
Culiacan Zoo	Albany	<i>Leopardis pardalis</i> (2), <i>Panthera leo</i> (1), <i>Felis concolor</i> (1), <i>Panthera tigris sumatrae</i> (1), <i>Panthera tigris tigris</i> (1), <i>Lynx rufus</i> (2), <i>Ursus americanus</i> (1), <i>Hippopotamus amphibius</i> (1), <i>Ara macao</i> (1), <i>Carassius auratus</i> (1), aquatic birds-feces (3), aquatic bird-soil (2), <i>Rattus</i> spp. (3), <i>Periplaneta americana</i> (3), <i>Musca domestica</i> (3), raw chicken(2)	
	(3,10, H: r:-)	<i>Hippopotamus amphibius</i> (1), <i>Bassariscus astutus</i> (1), aquatic birds-faeces (2), aquatic birds-water (1), <i>Cebus apella</i> (1)	
	San Diego	Aquatic birds-faeces (1), aquatic birds-soil (2), <i>Python regius</i> (1), <i>Python regius</i> (1), <i>Rattus rattus</i> (1)	
	Braenderup	<i>Mephitis macroura</i> (1), <i>Felis concolor</i> (1), <i>Panthera tigris</i> (2), <i>Procyon lotor</i> (1), <i>Ateles geoffroyi</i> (1)	
	Weltevreden	<i>Columba flavirostris</i> (1), <i>Columba fasciata</i> (1), <i>Sus scrofa domestica</i> (2), aquatic birds-faeces (1), aquatic birds-soil (1)	
	Derby	<i>Cebus apella</i> (1), <i>Panthera onca</i> (1), <i>Panthera tigris</i> (1), <i>Rattus rattus</i> (1)	
	Oranienburg	<i>Urocyon cinereoargenteus</i> (1), <i>Saimiri sciureus</i> (2)	
	(6,7, H: en x:-)	<i>Hippopotamus amphibius</i> (1), <i>Crocodylus acutus</i> (2)	
	Mazatlan Aquarium	Poona	Psittaciformes birds (3), <i>Rattus rattus</i> (2)
		Saintpaul	Aquatic birds-faeces (4)
Panama		<i>Crocodylus acutus</i> (1), <i>Rana</i> spp (1)	
Pomona		<i>Ramphastos sulfuratus</i> (1), biological filter (1)	
Newport		Aquatic birds-faeces (1)	
Enteritidis		Psittaciformes birds (1)	
Javiana		<i>Rana</i> spp (1)	
Give		<i>Iguana iguana</i> (1)	
Agona		<i>Ara</i> spp (1)	
sp		Aquatic birds-soil (1), <i>Crocodylus acutus</i> (1)	

MDR to four drugs in 54 isolates (DC, PE, AM and E, 65.1%), three drugs in 21 isolates (DC, PE, and E, 25.3%), six drugs in six isolates (AM, CF, CL, DC, E, and PE, 7.2%) and five drugs in two isolates (DC, PE, AM, E, and CF, 2.4%) were more frequently detected. MDR was observed in 11 *S. enterica* serovars isolated in this study with the greatest MDR in Weltevreden, followed by Give and Agona.

All *S. enterica* strains analysed in this study showed MDR, which corresponds with findings of others that cite antibiotic resistance as a very frequent phenomenon. MDR has become more common in *Salmonella enterica* similar to non-typhoidal salmonellosis, which has a high resistance rate to traditional antibiotics, possibly caused by their indiscriminate use in humans

and in animal feed [1].

The causes associated with antimicrobial resistance observed at both the zoo and the aquarium was multifactorial. We found that it was not unusual at both centres for medication to be administered based on incorrect diagnoses and, in many cases, administered incorrectly (causing inadequate bioavailability antimicrobials) due to the difficulty of administering antimicrobial treatments to wild animals. Together, these two issues promoted the antimicrobial resistance in the animals sampled.

Antimicrobials not only affect the target disease-causing pathogen, but also decimate the populations of sensitive microorganisms that exist throughout the body. This allows resistant strains to thrive and proliferate if necessary

Table 2. Antimicrobial resistance in *Salmonella* isolates from captive wild animals

<i>Salmonella</i> serovares	Total	AKK	AM	CF	CRO	CL	DC	ENX	E	GE	NET	PE	SXT
Derby	4	0	0	0	0	0	4	0	4	0	0	4	0
3,10, H: r:-	7	0	7	0	0	0	7	0	7	0	0	7	0
San Diego	6	0	6	0	0	0	6	0	6	0	0	6	0
Braenderup	6	0	6	0	0	0	6	0	6	0	0	6	0
6,7, H: en x:-)	3	0	3	0	0	0	3	0	3	0	0	3	0
Albany	28	0	28	0	0	0	28	0	28	0	0	28	0
Oranienburgg	3	0	3	0	0	0	3	0	3	0	0	3	0
Weltevreden	6	0	6	6	0	6	6	0	6	0	0	6	0
Poona	5	0	0	0	0	0	5	0	5	0	0	5	0
Saintpaul	4	0	0	0	0	0	4	0	4	0	0	4	0
Panama	2	0	0	0	0	0	2	0	2	0	0	2	0
Pomona	2	0	0	0	0	0	2	0	2	0	0	2	0
Newport	1	0	0	0	0	0	1	0	1	0	0	1	0
Enteritidis	1	0	0	0	0	0	1	0	1	0	0	1	0
Sp	2	0	0	0	0	0	2	0	2	0	0	2	0
Javiana	1	0	1	0	0	0	1	0	1	0	0	1	0
Give	1	0	1	1	0	0	1	0	1	0	0	1	0
Agona	1	0	1	1	0	0	1	0	1	0	0	1	0
Total	83	0	62	8	0	6	83	0	83	0	0	83	0
percentage of resistance	100	0	74.6	9.6	0	7.2	100	0	100	0	0	100	0

AK, amikacin; AM, ampicillin; CF, cephalothin; CRO, ceftriaxone; CL, chloramphenicol; DC, dicloxacillin; ENX, enoxacin; E, erythromycin; GE, gentamicin; NET, netilmicin; PE, penicillin; SXT, trimethoprim-sulfamethoxazole.

control measures are not implemented in cases of animals harbouring antimicrobial resistant organisms. These control measures include management facilities for isolation of infected animals and use of aseptic procedures and manual washing to prevent bacterial spread. As there is movement of microorganisms among veterinarians, the same animals or insects, the use of antimicrobials in animals carries risks for human beings that visit these parks, particularly children and the elderly as well as those who are high-risk or immunosuppressed [5]. In addition, it should be emphasized that with a high percentage of animals with excretory *Salmonella*, the probability of an intestinal genetic exchange between saprophytic microorganisms increases, causing more pathologic bacterial generation in this type of microenvironment [20]. *Salmonella* is

capable of surviving and multiplying in the soil for up to one year [20]. The factors that determine bacterial survival in soil include the following: type of soil, soil dryness, and populations of predatory organisms and the quantity of organic matter in the soil [10]. The environmental persistence of bacteria increases their continuous contact with antimicrobials and generates a natural selectivity that avoids attack [17]. Consistent with these results, serovars that showed the greatest antibacterial resistance were present in soil and faecal samples: Weltevreden, Give, and Agona, with six and five (Give and Agona) antimicrobial agents, respectively. The Weltevreden serovar was detected in six (9.5%) of the samples analysed, four from faeces and the soil of bird cages, similar to the results of others who identified antibiotic insensitivity in turtles,

wild birds, and aquarium fish [19]. The Give serovar was isolated from iguana faeces and the Agona serovar was isolated from macaw faeces. Herpetofauna have often been implicated as transmitters of *Salmonella* [12] the prevalence of *Salmonella* in reptile excretions ranges from 14% to 95% primarily in healthy captive reptiles [2,3], as in this study. Epidemiological studies have shown that birds constitute an important asymptomatic carrier and may transmit an infection transovarially to their progeny; this vertical transmission causes systemic spread and extensive faecal bacterial excretion resulting in environmental contamination that is difficult to eliminate [13].

Although the Typhimurium serovar is mostly cited as a pathogenic serovar that frequently shows resistance to multiple drugs [16], in the present study, other pathogenic serovars, including Weltevreden, Give, and Agona showed a greater degree of drug resistance. In contrast, Enteritidis serovar, which most frequently causes outbreaks of nutritional toxi-infection, was extremely sensitive to nearly all antibiotics except dicloxacillin, penicillin, and erythromycin. However, it has been argued that wild animals that are in contact with humans have increased prevalence of resistance to conventional antimicrobials used in their treatment [1].

The detection of MDR in strains analysed, even in serovars that rarely cause disease in captive wild animals, constitutes an important risk factor due to the great potential for genetic interchange between different bacterial genera in the intestine, leading to greater faecal excretion of the pathogen as well as the carriage state [4]. We observed this in our study, as the animals sampled did not display symptoms of any digestive disorder, illustrating one of the reasons why this bacterial genus represents a great risk of zoonotic transfer of resistance [18].

We conclude that MDR is common among *Salmonella* isolates that originate from wild animals in captivity in Sinaloa. It is unlikely that eradication of *Salmonella* in domestic and

wild animals will be possible in the foreseeable future. However, control and preventive measures aimed at reducing the accelerated growth of bacterial resistance in this zoonotic bacterial genus with a great impact on global public health needs to be intensified. The rational use of antimicrobials by well-informed veterinarians or by technicians trained in their use will also help minimize this issue of MDR *Salmonella*.

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