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Genotypic and Antibiogram Comparison of *Salmonella* spp. Isolates from Multiple Populations of Snakes in the Upper Midwest

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Crotalus viridis viridis (Prairie rattlesnake)

Pituophis catenifer sayi (Bull snake)

ABSTRACT

Background: Isolates of Salmonella are commonly found in the feces of reptiles as normal flora. This study examined isolates of Salmonella taken from populations of various populations of snakes in North Dakota, South Dakota, and Iowa for genetic relatedness, antimicrobial susceptibility, and the presence of the Yesinia high pathogenicity island (Y-HPI). Methods: 147 cloacal swab samples were taken from various populations and different species of snakes in the upper Midwest area. Multiple colonies were taken from each sample. All Salmonella isolated were analyzed for antimicrobial susceptibility, pulsed-field gel electrophoresis (PFGE) patterns, and PCR for the Y-HPI. The antimicrobial resistance patterns were obtained using the Kirby-Bauer method. All isolates were identified using Remel Rapid ID panels, PCR, and standard isolate identification methods. Results: Of the 147 swabs taken, approximately 66% were positive for Salmonella spp. After identification was completed, 143 Salmonella isolates were obtained. Twenty-two (15.3%) of the Salmonella isolates harbored resistance to one or more antibiotics. The PFGE patterns obtained varied by snake species and geographical location. Approximately half of the Salmonella isolates were positive for the Y-HPI. Conclusions: Isolates of Salmonella from the snakes sampled were generally sensitive to antibiotics. The presence of the Y-HPI did not correlate with the antimicrobial susceptibility of the isolates. In general, the PFGE patterns obtained indicate that isolates from specific populations and species of snakes were genetically similar. As these wild populations of snakes have no apparent exposure to antimicrobials, it is possible this explains the low percentage of resistant isolates.

METHODS

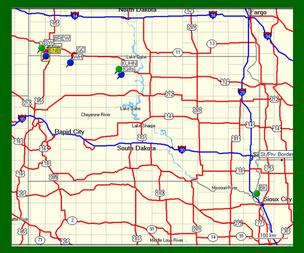
PFGE was performed according to the protocol described by the Center for Disease Control and Prevention[4] using the molecular size standard *Salmonella braenderup* BAA-664 (ATCC, Manassas, VA). Samples were further characterized using the BioNumerics software (Applied Maths, Austin, TX).

Antibiotic Resistance was determined by the Kirby-Bauer method using the BBL Sensi-disc susceptibility test discs (BD Diagnostic Systems, Franklin Lakes, NJ) with the following antibiotics: Ampicillin (AM), Amoxicillin/Clavulanic Acid (AmC), Amikacin (AN), Chloramphenicol (C), Ceftiofur (XNL), Sulfosoxazole (G), Kanamycin (K), Naladixic Acid (NA), Streptomycin (S), Tetracycline (TE), Gentamicin (GM), and Cephalothin (CF). Experimental antibiotics were chosen based on availability and cost. The *E. coli* strain 25944 (ATCC) was used as a positive control. Zone measurements were determined by the BIOMIC V[3] (Giles Scientific, Santa Barbara, CA).

PCR analysis for the Y-HPI was completed according to the methods previously described[1] using forward primer 5' → 3' GCGATGTTTAACCCCGATT and reverse primer 5' → 3' TGCCTGGAAACCCTGAGACT. DNA was extracted by inoculating *Salmonella* isolates on MacConkey agar plates and incubating overnight at 37ºC. A single colony of each isolate was added to 40 µL of TE buffer with 1% 20 mg/mL proteinase K. Samples were incubated at 55ºC for 10 min followed by a 10 min incubation at 80ºC. DNA was then diluted with 80 µL of sterile water, centrifuged for 5 min, and stored at -20ºC for PCR analysis.

Cloacal swabs were taken from different species of snakes in South Dakota, North Dakota, and Iowa using sterile cotton swabs that were kept moist until culturing.

1



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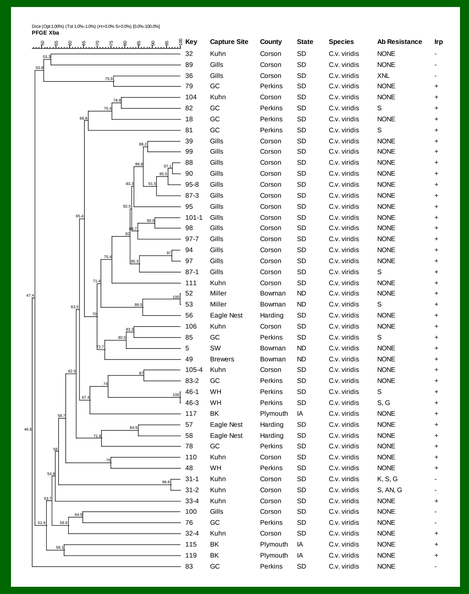
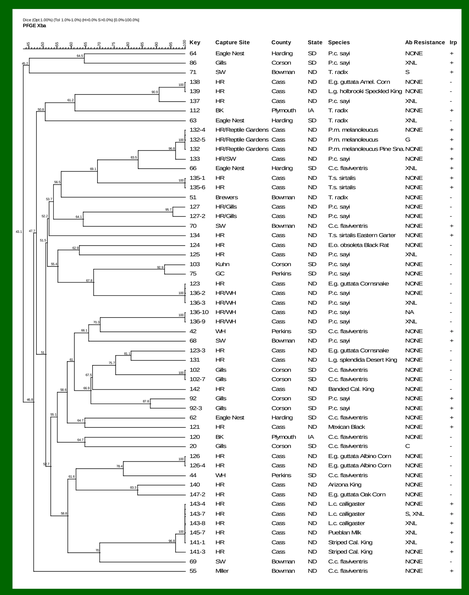
Figures 1 and 2: A map of locations where snakes were sampled.



Key	Capture Site	County	State	Species	Ab Resistance	Irp
32	Kuhn	Corson	SD	C.v. viridis	NONE	-
89	Gills	Corson	SD	C.v. viridis	NONE	-
36	Gills	Corson	SD	C.v. viridis	XNL	-
79	GC	Perkins	SD	C.v. viridis	NONE	+
104	Kuhn	Corson	SD	C.v. viridis	NONE	+
82	GC	Perkins	SD	C.v. viridis	S	+
18	GC	Perkins	SD	C.v. viridis	NONE	+
81	GC	Perkins	SD	C.v. viridis	S	+
39	Gills	Corson	SD	C.v. viridis	NONE	+
99	Gills	Corson	SD	C.v. viridis	NONE	+
88	Gills	Corson	SD	C.v. viridis	NONE	+
90	Gills	Corson	SD	C.v. viridis	NONE	+
95-8	Gills	Corson	SD	C.v. viridis	NONE	+
87-3	Gills	Corson	SD	C.v. viridis	NONE	+
95	Gills	Corson	SD	C.v. viridis	NONE	+
101-1	Gills	Corson	SD	C.v. viridis	NONE	+
98	Gills	Corson	SD	C.v. viridis	NONE	+
97-7	Gills	Corson	SD	C.v. viridis	NONE	+
94	Gills	Corson	SD	C.v. viridis	NONE	+
97	Gills	Corson	SD	C.v. viridis	NONE	+
87-1	Gills	Corson	SD	C.v. viridis	S	+
111	Kuhn	Corson	SD	C.v. viridis	NONE	+
52	Miller	Bowman	ND	C.v. viridis	NONE	+
53	Miller	Bowman	ND	C.v. viridis	S	+
56	Eagle Nest	Harding	SD	C.v. viridis	NONE	+
106	Kuhn	Corson	SD	C.v. viridis	NONE	+
85	GC	Perkins	SD	C.v. viridis	S	+
5	SW	Bowman	ND	C.v. viridis	NONE	+
49	Brewers	Bowman	ND	C.v. viridis	NONE	+
105-4	Kuhn	Corson	SD	C.v. viridis	NONE	+
83-2	GC	Perkins	SD	C.v. viridis	NONE	+
46-1	WH	Perkins	SD	C.v. viridis	S	+
46-3	WH	Perkins	SD	C.v. viridis	S, G	+
117	BK	Plymouth	IA	C.v. viridis	NONE	+
57	Eagle Nest	Harding	SD	C.v. viridis	NONE	+
58	Eagle Nest	Harding	SD	C.v. viridis	NONE	+
78	GC	Perkins	SD	C.v. viridis	NONE	+
110	Kuhn	Corson	SD	C.v. viridis	NONE	+
48	WH	Perkins	SD	C.v. viridis	NONE	+
31-1	Kuhn	Corson	SD	C.v. viridis	K, S, G	-
31-2	Kuhn	Corson	SD	C.v. viridis	S, AN, G	-
33-4	Kuhn	Corson	SD	C.v. viridis	NONE	+
100	Gills	Corson	SD	C.v. viridis	NONE	-
76	GC	Perkins	SD	C.v. viridis	NONE	-
32-4	Kuhn	Corson	SD	C.v. viridis	NONE	+
115	BK	Plymouth	IA	C.v. viridis	NONE	+
119	BK	Plymouth	IA	C.v. viridis	NONE	+
83	GC	Perkins	SD	C.v. viridis	NONE	-

Figure 3: Dendrogram of the *Salmonella* spp. isolates from prairie rattlesnake isolates.



Key	Capture Site	County	State	Species	Ab Resistance	Irp
64	Eagle Nest	Harding	SD	P.c. sayi	NONE	+
86	Gills	Corson	SD	P.c. sayi	XNL	+
71	SW	Bowman	ND	T. radix	S	+
138	HR	Cass	ND	E.g. guttata Amel. Corn	NONE	-
139	HR	Cass	ND	L.g. holbrooki Speckled King	NONE	-
137	HR	Cass	ND	P.c. sayi	XNL	-
112	BK	Plymouth	IA	T. radix	NONE	+
63	Eagle Nest	Harding	SD	T. radix	XNL	-
132-4	HR/Reptile Gardens	Cass	ND	P.m. melanoleucus	NONE	+
132-5	HR/Reptile Gardens	Cass	ND	P.m. melanoleucus	G	+
132	HR/Reptile Gardens	Cass	ND	P.m. melanoleucus Pine Sna	NONE	+
133	HR/SW	Cass	ND	P.c. sayi	NONE	+
66	Eagle Nest	Harding	SD	C.c. flaviventris	XNL	+
135-1	HR	Cass	ND	T.s. sirtalis	NONE	+
135-6	HR	Cass	ND	T.s. sirtalis	NONE	+
51	Brewers	Bowman	ND	T. radix	NONE	-
127	HR/Gills	Cass	ND	P.c. sayi	NONE	-
127-2	HR/Gills	Cass	ND	P.c. sayi	NONE	-
70	SW	Bowman	ND	C.c. flaviventris	NONE	+
134	HR	Cass	ND	T.s. sirtalis Eastern Carter	NONE	+
124	HR	Cass	ND	E.o. obsoleta Black Rat	NONE	-
125	HR	Cass	ND	P.c. sayi	XNL	-
103	Kuhn	Corson	SD	P.c. sayi	NONE	-
75	GC	Perkins	SD	P.c. sayi	NONE	-
123	HR	Cass	ND	E.g. guttata Cornsnake	NONE	-
136-2	HR/WH	Cass	ND	P.c. sayi	NONE	-
136-3	HR/WH	Cass	ND	P.c. sayi	XNL	-
136-10	HR/WH	Cass	ND	P.c. sayi	NA	-
136-9	HR/WH	Cass	ND	P.c. sayi	XNL	-
42	WH	Perkins	SD	C.c. flaviventris	NONE	+
68	SW	Bowman	ND	P.c. sayi	NONE	+
123-3	HR	Cass	ND	E.g. guttata Cornsnake	NONE	-
131	HR	Cass	ND	L.g. splendida Desert King	NONE	-
102	Gills	Corson	SD	C.c. flaviventris	NONE	-
102-7	Gills	Corson	SD	C.c. flaviventris	NONE	-
142	HR	Cass	ND	Banded Cal. King	NONE	-
92	Gills	Corson	SD	P.c. sayi	NONE	+
92-3	Gills	Corson	SD	P.c. sayi	NONE	+
62	Eagle Nest	Harding	SD	C.c. flaviventris	NONE	+
121	HR	Cass	ND	Mexican Black	NONE	+
120	BK	Plymouth	IA	C.c. flaviventris	NONE	-
20	Gills	Corson	SD	C.c. flaviventris	C	-
126	HR	Cass	ND	E.g. guttata Albino Corn	NONE	-
126-4	HR	Cass	ND	E.g. guttata Albino Corn	NONE	-
44	WH	Perkins	SD	C.c. flaviventris	NONE	-
140	HR	Cass	ND	Arizona King	NONE	-
147-2	HR	Cass	ND	E.g. guttata Oak Corn	NONE	-
143-4	HR	Cass	ND	L.c. calligaster	NONE	+
143-7	HR	Cass	ND	L.c. calligaster	S, XNL	+
143-8	HR	Cass	ND	L.c. calligaster	XNL	+
145-7	HR	Cass	ND	Pueblan Milk	NONE	+
141-1	HR	Cass	ND	Striped Cal. King	XNL	+
141-3	HR	Cass	ND	Striped Cal. King	NONE	+
69	SW	Bowman	ND	C.c. flaviventris	NONE	-
55	Miller	Bowman	ND	C.c. flaviventris	NONE	+

Figure 4: Dendrogram of the *Salmonella* spp. isolates from all other species of snakes.

Map Name	Full Description	Location Description	County	State
BK	Broken Kettle Grasslands	North of Sioux City, IA	Plymouth	IA
BREW	Brewers pasture	NW of Bowman, ND	Bowman	ND
ENB	Eagle Nest Butte	South of Bowman, ND	Harding	SD
GC	Public pasture by golf course	just south of Hettinger, ND	Perkins	SD
Gills	Larry Gill's ranch	NE of Timberlake, SD	Corson	SD
KUHN	Kuhn's pasture land	NE of Timberlake, SD	Corson	SD
Miller	Chad Miller's pasture	SW of Bowman, ND	Bowman	ND
SW	Steve Weigum's pasture	SW of Bowman, ND	Bowman	ND
WH	Parker's pasture land	SW of Lodgepole, SD	Perkins	ND
HR	NDSU Herp Room	Fargo, ND	Cass	ND

Table 1. Legend for PFGE charts

RESULTS AND DISCUSSION

The results are shown in Figures 3 and 4. The *Yersinia* high pathogenicity island was more commonly found in the Prairie rattle snake, but was also found in *Salmonella* isolates from other species of snakes. The Y-HPI was not associated with any specific location or other species of snakes. The vast majority of isolates from all species of snakes were susceptible to most antibiotics tested; however, the most common resistance was to ceftiofur. The genetic relatedness, as based on PFGE, showed that while some isolates taken from snakes in the same general geographic location were highly related and that they were not exclusive to the species of snake. In the prairie rattlesnakes, two isolates from the Miller capture site, and two from Parker's pastureland were 100% identical. In some cases, identical isolates were found in multiple species of snake in the same capture site or location. In some cases, the presence of antibiotic resistances did not affect PFGE patterns.

CONCLUSIONS

***Salmonella* are commonly found in reptiles, including snakes.[2] This research has shown that the Y-HPI is widely spread in many species of snakes found in ND, IA, and SD. It is tempting to speculate that as snakes in captivity or in the wild are not normally exposed to antibiotics, thus the lack of selective pressure could allow for more susceptible isolates. While some snakes, regardless of species, may share genetically identical or nearly identical *Salmonella* isolates, there is frequently genetic variability among *Salmonella* isolates even within the same population of snakes.**

References

1) **Bach, S., A. Almeida, and E. Carniel. The *Yersinia* high-pathogenicity island is present in different members of the family *Enterobacteriaceae*.**

2) **Mermin J, Hoar B, Angulo FJ. Iguanas and *Salmonella* Marina infection in children: a reflection of the increasing incidence of reptile-associated salmonellosis in the United States. Pediatrics 1997;99:399-402.**

Acknowledgements

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