

Phenotypic and Genotypic Characterization of Antibiotic Resistance Integrons in *Salmonella* *enterica* serovar Newport

Raymond Soto, Department of Microbiology;

Faculty Mentor: Dr. John D. Willford, Departments of
Microbiology and Molecular Biology;

Graduate Mentor: Ashley Driscoll, Department of Petroleum
and Chemical Engineering

Salmonella Introduction

- ◉ *Salmonella enterica*

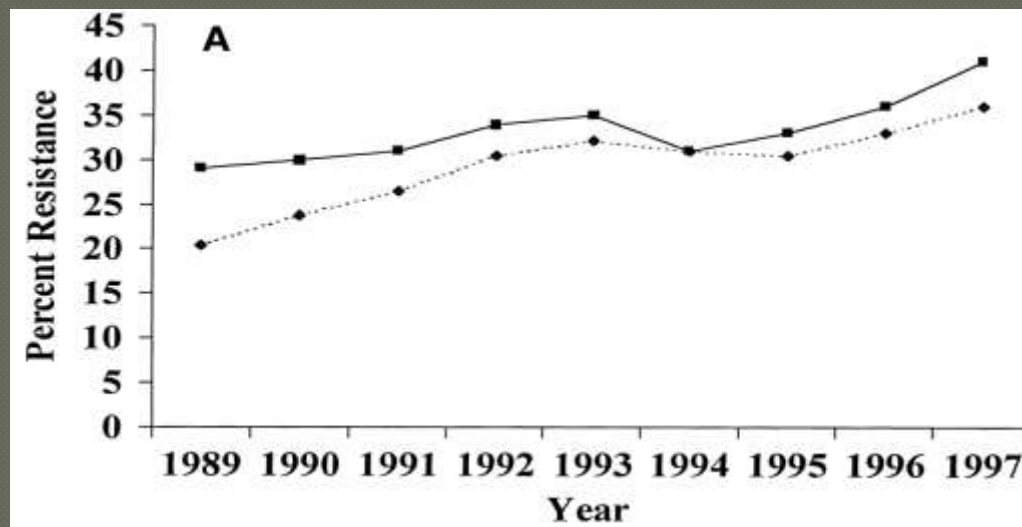
- ◉ Infection

- Diarrhea
- Fever
- Cramps
 - Supportive Treatment
- Sepsis
 - Antibiotic use required
- Around 550 food-borne related deaths per year (Mead et al. 1999)

Antibiotic Resistance

- What does it mean?
- How often does it occur?
 - Resistance rates on the rise

Patients Infected with Methicillin Resistant *Staphylococcus aureus*



(Fridkin et al, 1999)

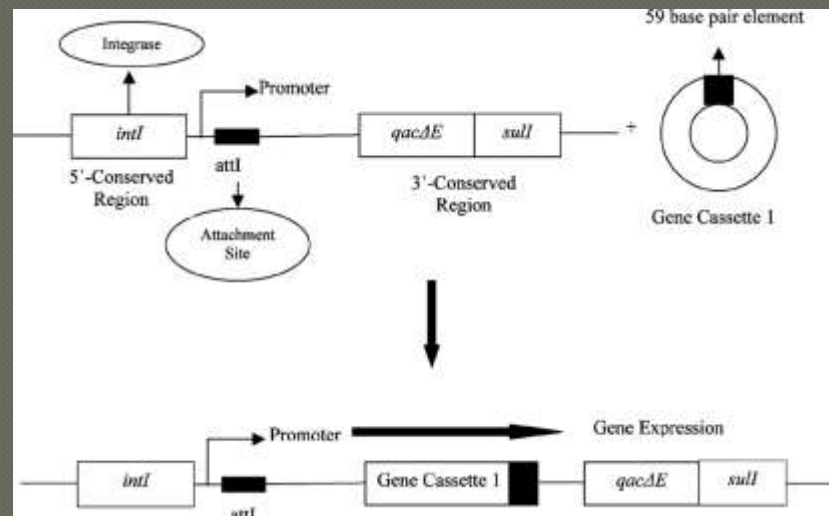
Antibiotic Resistance

- What causes Antibiotic Resistance?
 - Selective Pressure
 - Over prescription of antibiotics
 - Misuse of antibiotics
 - Emphasis on development of chronic medicine rather than antibiotics
 - Naturally occurring
 - Lateral gene transfer and mutations
 - Plasmids
 - Integrons

Integrans

What is an Integrans?

- Mobile DNA
 - Random genome insertion
- Conserved Sequences (CS)
- Genes Cassettes Inserted Between CS



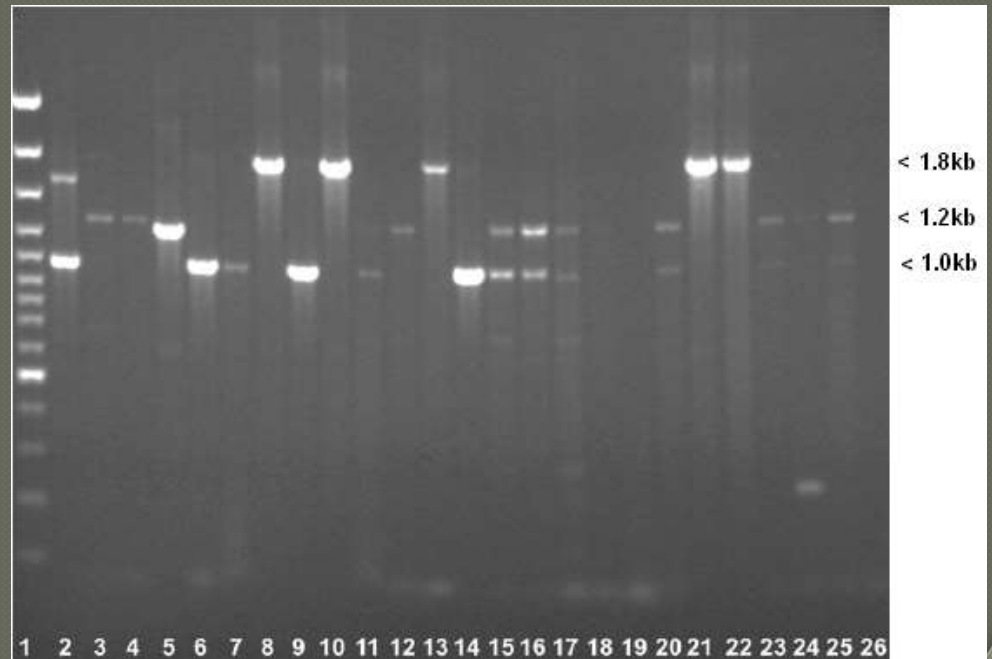
(Harbottle et al. 2006)

Previous Study

	SN1	SN2	SN3	SN4	SN5	SN6	SN7	SN8	SN9	SN10	SN11	SN12	SN13	SN14	SN15	SN16	SN17
Cephazolin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Penicillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Polymyxin B	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cephalothin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Ampicillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Amikacin	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S
Sulfamethazole W/ Trimeth	R	S	S	R	I	S	R	S	R	S	S	R	S	S	S	S	S
Tetracycline	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Streptomycin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Naladixic Acid	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S
Gentamicin	S	S	S	R	R	S	S	R	R	S	S	S	R	S	S	S	S
Erythromicin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Neomicine	R	S	S	R	S	I	S	R	R	I	I	S	I	R	S	R	S

Previous Study

- ◉ *Salmonella enterica* serovar Newport
- ◉ Uncharacterized Integrons
 - 1.0, 1.2, and 1.8 kb



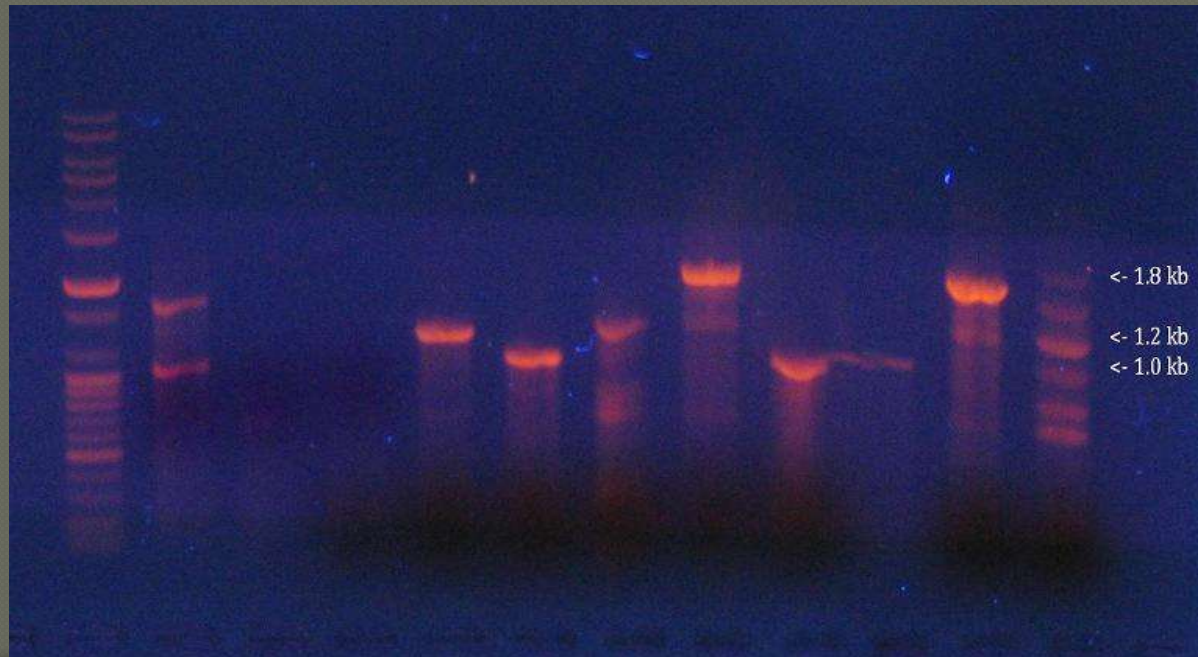
(Willford et al. 2007)

Research Objectives

- Clone previously identified 1.0, 1.2, and 1.8kb integron gene cassettes
- Characterize cloned integrons for antibiotic resistance phenotypically and genotypically
 - Individually
 - In combination

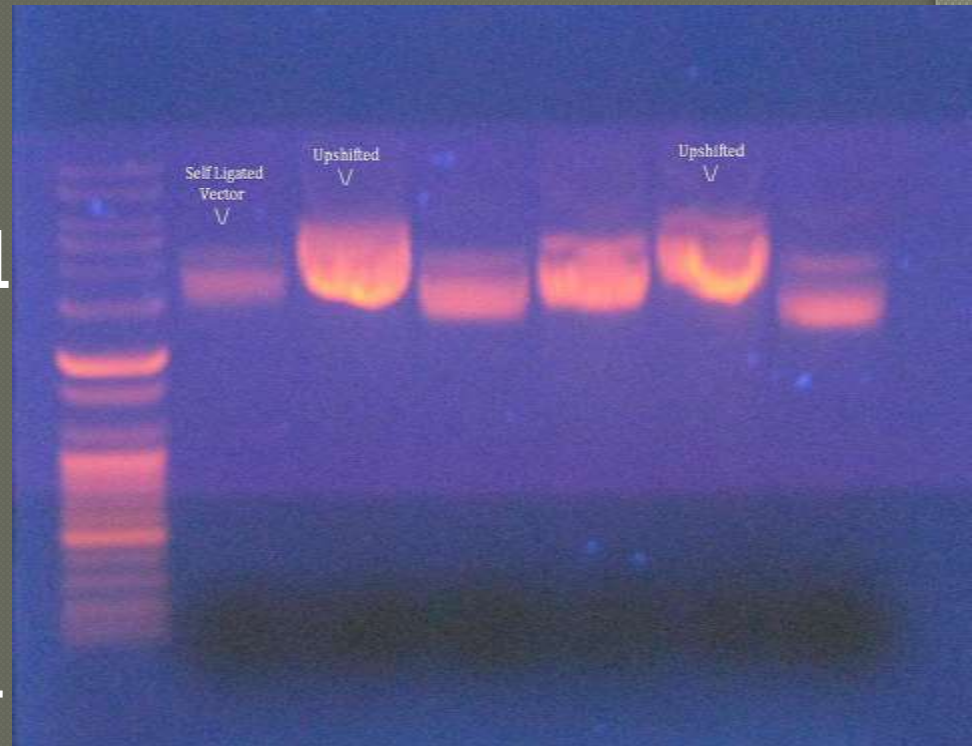
Procedure

- Determined antibiotic resistance profiles for *S. Newport* strains and control strains
- Isolated bacterial DNA
- Amplified integrons using PCR



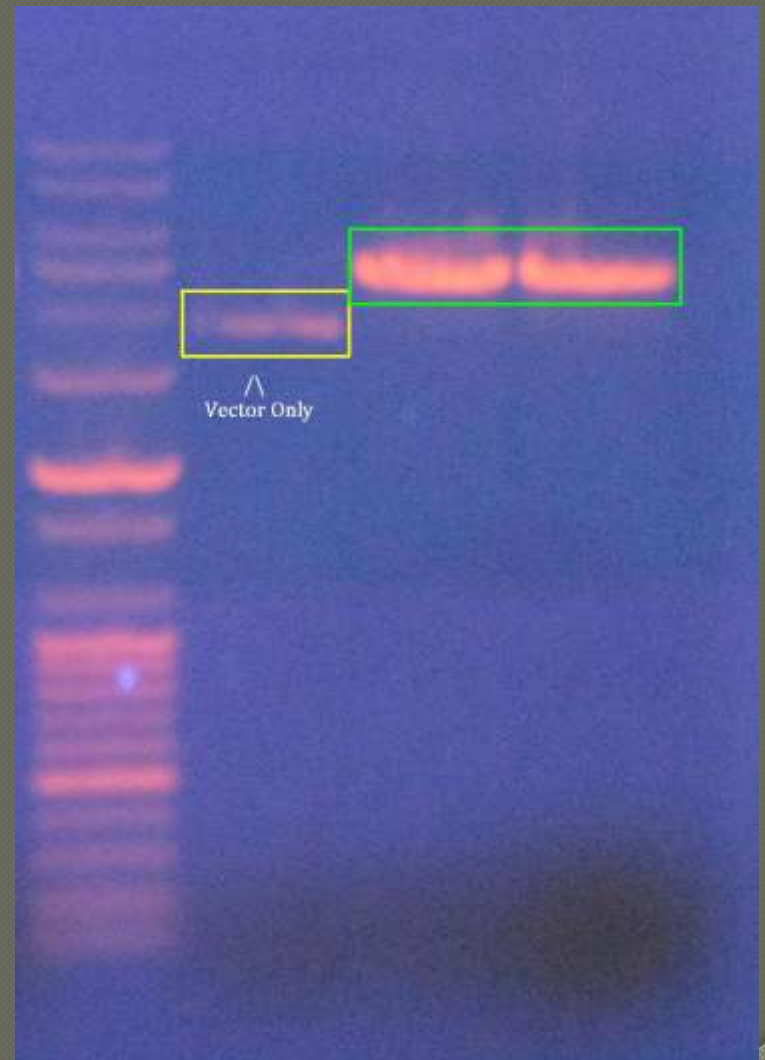
Procedure

- Ligated and transformed Integron PCR DNA with pET vector and later with pST Vector
- Screened DNA from transformants using Plasmid Prep Kit I



Procedure

- Integron containing plasmids sent to Nucleic Acid Exploration Facility (NAEF) at the University of Wyoming
- Sequences screened using BLAST to find matching genes



Results

NCBI BlastNucleotide Sequence (1094 letters) - Windows Internet Explorer provided by University of Wyoming

http://blast.ncbi.nlm.nih.gov/Blast.cgi

NCBI BlastNucleotide Sequence (1094 letters)

Legend for links to other resources: UniGene GEO Gene Structure Map Viewer

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E-value	Max ident	Links
AC143524.1	AadA1 [Escherichia coli]	549	549	76%	2e-154	99%	
ZP_03063004.1	streptomycin 3'-adenylyltransferase [Escherichia coli B171] >embI	549	549	76%	2e-154	99%	
AF967771.1	AadA [Escherichia coli APEC O1]	549	549	76%	2e-154	99%	G
AA828672.1	aminoglycoside adenylyltransferase [Integration vector pCO13PKS] :	549	549	76%	2e-154	99%	
AA678957.1	streptomycin/spectinomycin adenylyltransferase [Binary vector pLHR	549	549	76%	2e-154	99%	
CAA26199.1	put. AAD (31/9) precursor [Escherichia coli]	549	549	76%	2e-154	99%	
AA193764.1	AadA [Integration vector pK202]	549	549	76%	2e-154	99%	
AB488964.1	aminoglycoside-3'-adenylyltransferase [Vibrio cholerae]	548	548	76%	6e-154	99%	
YP_001144148.1	aminoglycoside resistance protein [Aeromonas salmonicida subsp. i	548	548	76%	6e-154	99%	G
ZP_01974653.1	AadA [Vibrio cholerae 033] >gb BAZ77746.1 AadA [Vibrio cholerae]	546	546	76%	1e-153	99%	
BA558662.1	aadA1c [Vibrio cholerae] >gb ACN50165.1 AadA1c [Vibrio cholerae]	546	546	76%	1e-153	99%	
AAK97736.1	spectinomycin adenylyltransferase [Binary vector pP2F 25S Hislac-att	546	546	76%	2e-153	99%	
ACN50164.1	AadA1c [Vibrio cholerae]	545	545	76%	3e-153	98%	
AA693750.1	aminoglycoside-3'-adenylyltransferase [synthetic construct]	545	545	76%	4e-153	98%	
YP_001102017.1	aminoglycoside resistance protein [Salmonella enterica subsp. ente	533	535	76%	5e-150	96%	G
YP_001715257.1	aminoglycoside resistance protein [Acinetobacter baumannii AYE] >	529	529	73%	3e-148	99%	G
AC146476.1	aminoglycoside 3'-adenylyltransferase/c-myc 9E10 epitope fusion [519	519	72%	2e-145	100%	
HF_862002.1	aminoglycoside resistance protein [Escherichia coli] >ref NP_05389	519	519	72%	2e-145	100%	G
CAA08944.1	aminoglycoside adenylyltransferase [Salmonella typhimurium] >emb	518	518	72%	4e-145	99%	
YP_128852.1	aminoglycoside resistance protein [uncultured bacterium] >ref YP_1	518	518	72%	5e-145	99%	G
AB201795.1	aadA1a [Escherichia coli]	518	518	72%	6e-145	99%	
AA191745.1	ANT(3)-1 [Acinetobacter baumannii]	518	518	72%	6e-145	99%	
AA649781.1	aminoglycoside 3'-(9)-O-adenylyltransferase [Escherichia coli] >em	518	518	72%	6e-145	99%	
BAF83887.1	aminoglycoside-3'-adenylyltransferase [uncultured bacterium]	517	517	72%	8e-145	99%	
AB429106.1	AAD(3') aminoglycoside (3')-adenylyltransferase [Riemerella anat	517	517	72%	8e-145	99%	
AAK14202.1	aminoglycoside 3'-adenylyltransferase [Acinetobacter genomosp. 3'	517	517	72%	8e-145	99%	
AA855010.1	aminoglycosideadeninetransferase [Chloroplast transformation vect	517	517	71%	8e-145	100%	
CA042867.1	aminoglycoside adenylyltransferase [Salmonella typhimurium]	517	517	72%	8e-145	99%	
CA120142.1	aminoglycoside 3'-adenylyltransferase [Salmonella enterica]	517	517	72%	1e-144	99%	
CA846709.1	aminoglycoside adenylyltransferase [Acinetobacter baumannii] >em	517	517	72%	1e-144	99%	
AB544429.1	aminoglycoside adenylyltransferase [Salmonella enterica subsp. em	517	517	72%	1e-144	99%	
AA814532.1	aminoglycoside adenine transferase [Chloroplast transformation ve	517	517	72%	1e-144	99%	
BA038867.1	aminoglycoside adenylyltransferase [Escherichia coli]	517	517	72%	1e-144	99%	
ZP_04298140.1	streptomycin 3'-O-adenylyltransferase [Vibrio cholerae 033] >gb F	517	517	72%	1e-144	99%	
AB029667.1	AadA1 [Salmonella enterica subsp. enterica serovar Virchow]	516	516	72%	1e-144	99%	
ARH07980.1	aminoglycoside 3'-adenylyltransferase [Escherichia coli]	516	516	72%	1e-144	99%	
AAH1424.1	aminoglycoside-3'-adenylyltransferase [uncultured bacterium]	516	516	72%	1e-144	99%	
AB832514.1	AAD [Plasmid transformation vector pPRV11DL] >gb ABK01315.1 st	516	516	72%	1e-144	99%	
ACX94065.1	aminoglycoside 3'-adenylyltransferase [Acinetobacter genomosp. 3]	516	516	72%	2e-144	99%	
ARP35560.1	AadA1 [Escherichia coli]	516	516	72%	2e-144	99%	
AB076950.1	aminoglycoside-3'-adenylyltransferase [uncultured bacterium]	516	516	72%	2e-144	99%	

Results

- 1.0 kb integron successfully cloned
- Identification of adenylyltransferase gene conferring aminoglycoside resistance
 - Specifically to streptomycin
- Altered antibiotic resistance profile observed

Results

	pET24	pET10S13 # 1	pET10S13 # 4
Cephazolin	S	S	S
Penicillin	R	R	R
Polymyxin B	S	S	S
Cephalothin	R	R	R
Ampicillin	R	R	R
Amikacin	S	S	S
Sulfamethazole w/ Trimethoprim	S	S	S
Tetracycline	R	R	R
Streptomycin	S	R	R
Naladixic Acid	I	R	R
Gentamycin	S	S	S
Erythroycin	R	S	I
Neomicine	S	S	S
Amoxicillin w/ Clauvanic Acid	S	S	S
Chloramphenicol	S	S	S
Cefoxitin	S	S	S

Results

NCBI Blast:Nucleotide Sequence (937 letters) - Windows Internet Explorer provided by University of Wyoming

http://blast.ncbi.nlm.nih.gov/Blast.cgi

[Favorites](#)
[Parallel Universe Across th...](#)
[Pandora Radio - Listen to ...](#)
[NCBI Blast:Nucleotide ...](#)
[How to Take a Screenshot ...](#)

Descriptions

Legend for links to other resources: [U](#) UniGene [G](#) GEO [G](#) Gene [S](#) Structure [M](#) Map Viewer

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
YP_001745373.1	dihydrofolate reductase type 1 (dihydrofolate reductase type 1) (trv Dhfr1 (Klebsiella pneumoniae) >ob ABK80042.1 Dhfr1 (Escherichia coli) >ob ACL98104.1) dhvdr:	317	317	50%	4e-85	99%	G
ABF48384.1	dihydrofolate reductase (Escherichia coli) >ob ACL98104.1) dhvdr:	315	315	50%	9e-85	98%	
ACL98085.1	dihydrofolate reductase type 1 (Salmonella sp. D78)	315	315	50%	1e-84	98%	
ACI22953.1	trimethoprim-resistant dihydrofolate reductase (Escherichia coli)	312	312	50%	1e-83	97%	
CAG27847.1	dihydrofolate reductase type V (Moraxella sp. TR-90)	312	351	69%	1e-83	100%	
AAA26061.1	dihydrofolate reductase (Plasmid R483)	310	310	48%	5e-83	100%	
YP_001746867.1	dihydrofolate reductase (Salmonella enterica subsp. enterica serovar enterica)	308	308	48%	1e-82	99%	G
AAH72080.1	dihydrofolate reductase (uncultured bacterium) >ob ACI22923.1 d	308	308	48%	2e-82	98%	
ACI85217.1	dihydrofolate reductase (Vibrio cholerae)	302	303	47%	5e-81	100%	
CAI78823.1	dihydrofolate reductase type 1 (Providencia sp. TR 58)	296	296	47%	8e-79	99%	
CAR65887.1	dihydrofolate reductase (Escherichia coli)	294	294	50%	3e-78	91%	
AAF22229.1	dihydrofolate reductase DHFRXvb (Klebsiella pneumoniae)	291	291	50%	2e-77	89%	
AAK33556.1	dihydrofolate reductase (Aeromonas salmonicida subsp. salmonicida)	261	261	49%	2e-68	80%	
CAI29476.1	dihydrofolate reductase type V (Enterobacter sp. TR 85) >ob ABAS-	249	249	50%	1e-64	74%	
BAD07285.1	dihydrofolate reductase (Salmonella enterica subsp. enterica serovar enterica)	238	271	56%	1e-64	100%	
ACI22851.1	dihydrofolate reductase (Escherichia coli)	246	248	50%	1e-64	74%	
AAN41406.1	dihydrofolate reductase (uncultured bacterium)	246	246	50%	5e-64	73%	
BAI50911.1	dihydrofolate reductase (Moritella japonica)	245	245	49%	2e-63	76%	
YP_001740004.1	dihydrofolate reductase type 5 (Escherichia coli: SMS-3-5) >emb CA	241	241	50%	3e-62	71%	G
ACI22910.1	dihydrofolate reductase-like protein (Escherichia coli) >ob ACI2292	230	262	59%	4e-62	100%	
ABR77358.1	dihydrofolate reductase (Salmonella enterica subsp. enterica serovar enterica)	239	239	50%	8e-62	71%	
CAP69468.1	dihydrofolate reductase (Escherichia coli) >emb CAT00043.1 dhv:	238	238	50%	2e-61	71%	
AAN41422.1	dihydrofolate reductase (uncultured bacterium) >ob ACI22956.1 d	238	238	50%	2e-61	70%	
ARI24166.1	dihydrofolate reductase (Escherichia coli)	234	234	50%	2e-60	68%	
AAN41406.1	dihydrofolate reductase (uncultured bacterium) >ob AAN41410.1 d	234	234	50%	2e-60	68%	
ACT52963.1	dihydrofolate reductase (Salmonella enterica)	233	233	50%	6e-60	67%	
CAP69466.1	dihydrofolate reductase (Escherichia coli)	233	233	50%	8e-60	67%	
CPH31027.1	dihydrofolate reductase (Escherichia coli)	232	232	50%	1e-59	69%	
CAP64347.1	dihydrofolate reductase type V (Escherichia coli)	226	226	46%	7e-58	73%	
CAP72500.1	dihydrofolate reductase (uncultured bacterium)	218	218	33%	2e-55	97%	
CAQ11597.1	dihydrofolate reductase (Escherichia coli)	218	218	47%	6e-55	69%	
CAV19087.1	Dihydrofolate reductase type I (trimethoprim resistance) (Vibrio sp)	215	215	50%	2e-54	64%	
ACP16552.1	dihydrofolate reductase (Vibrio fluvialis)	209	209	50%	9e-53	61%	G
CAP72501.1	dihydrofolate reductase (uncultured bacterium)	191	223	45%	2e-50	100%	
BAD23062.1	hypothetical protein (Salmonella sp. H16-244) >ob AAAY87123.1 un	144	244	32%	2e-50	100%	
CAI30961.2	dihydrofolate reductase type 17 (Serratia sp. TR48)	195	195	41%	2e-48	60%	
CAG27848.1	hypothetical protein (Moraxella sp. TR-90)	109	211	29%	3e-34	97%	
ZF_0480422.1	Dihydrofolate reductase (Clostridium cellulovorans 7438) >ob EES2	110	110	49%	8e-23	39%	

Results

- 1.2 kb integron sequece was sequenced and coded for Dihydrofolate Reductase conferring Trimethoprim resistance (Laroche, Pawlak , Berthe, Skurnik , and Petit 118-130)
- Conferred antibiotic resistance was confirmed with antibiotic testing

	EC	pET24	pE12S4-29
Trimethoprim plate (TSA with 5µg/ml of Trimethoprim)	NG	NG	G

Brief Conclusions

- 1.0 kb integron conferred antibiotic resistance to Streptomycin
- 1.2 kb integron conferred antibiotic resistance to Trimethoprim
- 1.8 kb integron could not be successfully cloned

Future Work

- Cloning of 1.8 kb integron
- Sequencing of 1.8 kb integron
- Transformation of all three integrons in combination, i.e. 1.0 and 1.2, all three etc.
 - Establishment on antibiotic resistance profiles for combined transformants

References

1. Fridkin, S. K., Gaynes R. P. "Antimicrobial Resistance in Intensive Care Units." *Clinics in Chest Medicine* 20(1999): 303-316.
2. Harbottle, H., Thakur, S., Zhao, S. and White, D. G. "Genetics of Antimicrobial Resistance", *Animal Biotechnology* 17(2006):111 — 124.
3. Willford, J, Manley, W, Rebelin, T, Goodridge, L.. "Pulsed Field Ribotype and Integron Analysis of Multidrug Resistant Isolates of *Salmonella enterica* serovar Newport." *Food Research International* 40(2007): 975-981.
4. Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M., Tauxe, R. V.. "Food-Related Illness and Death in the United States." *Emerging Infectious Diseases* 5(1999):607-625
5. Laroche, Emile, Barbara Pawlak , Thierry Berthe, David Skurnik , and Fabienne Petit . "Occurrence of antibiotic resistance and class 1, 2 and 3 integrons in *Escherichia coli* isolated from a densely populated estuary (Seine, France)." *FEMS Microbiology Ecology*. 68.1 (2009): 118-130

Acknowledgements

- ◉ McNair Scholars Program
- ◉ EPSCoR
- ◉ INBRE
- ◉ Zackie Salmon
- ◉ Susan Stoddard
- ◉ Dr. Gerry Andrews
- ◉ NAEF, Terry McClean
- ◉ MOLB Teaching Lab, Karen White

Questions



<http://www.cartoonstock.com/newscartoons/cartoonists/mba/lowres/mban6951.jpg>