

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

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Salmonella Introduction

- *Salmonella enterica*

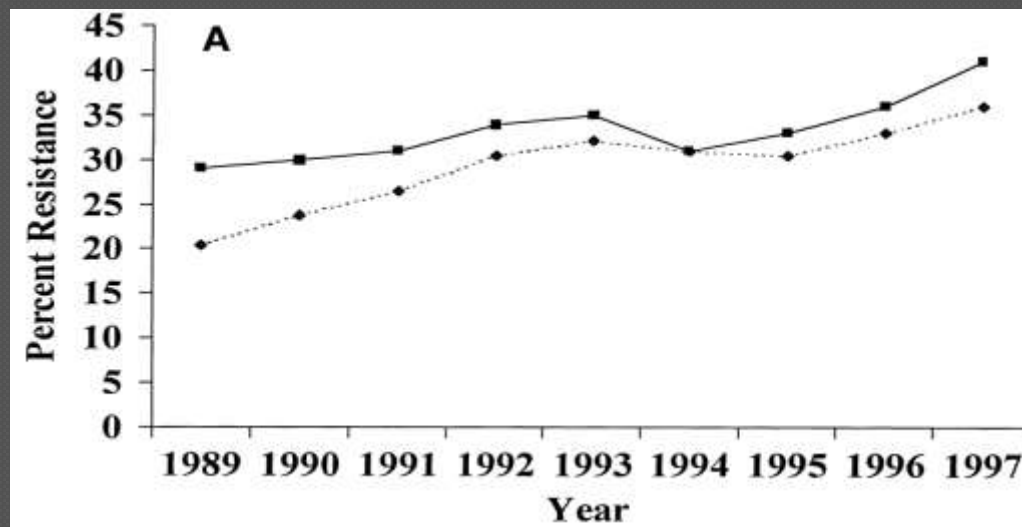
- Infection

- 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)

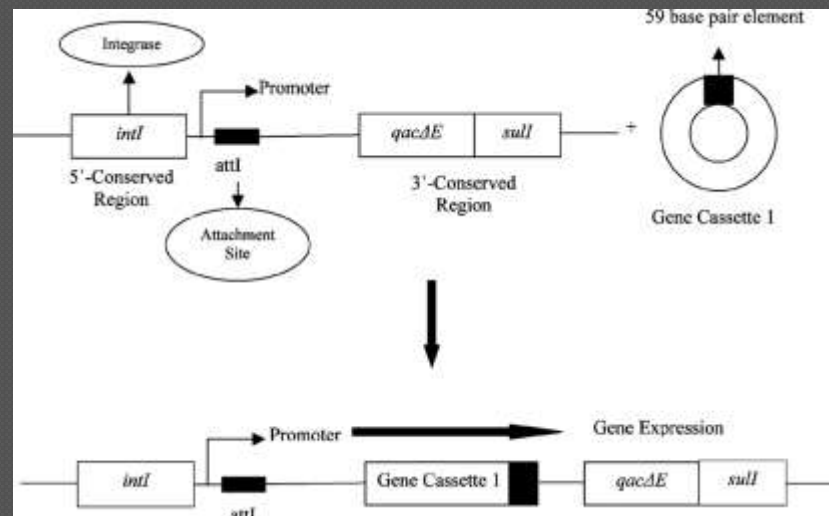
Antibiotic Resistance

- 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



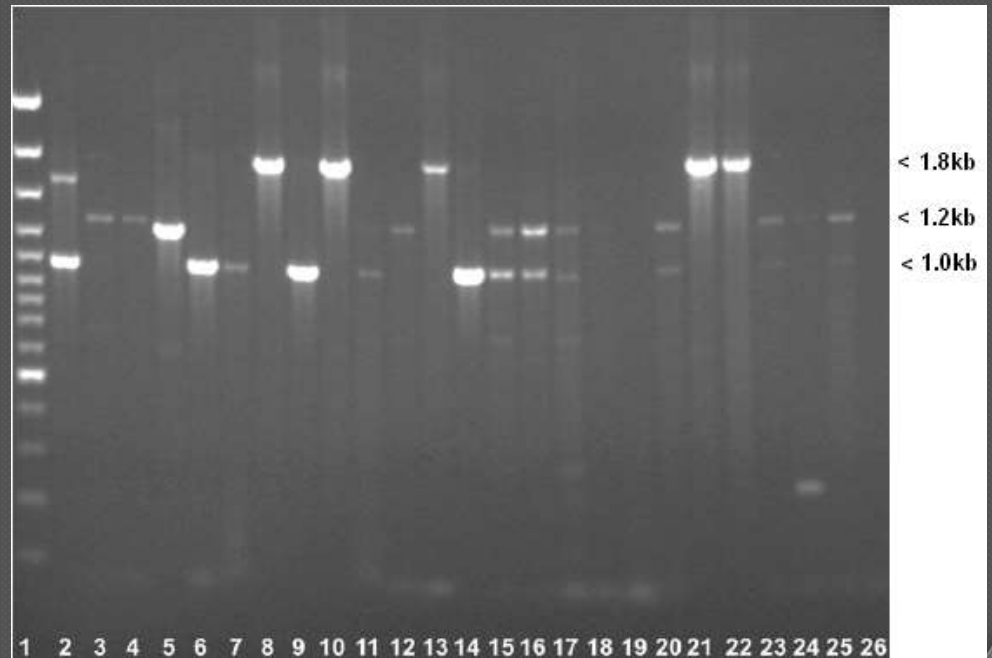
(Harbottle et al. 2006)

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

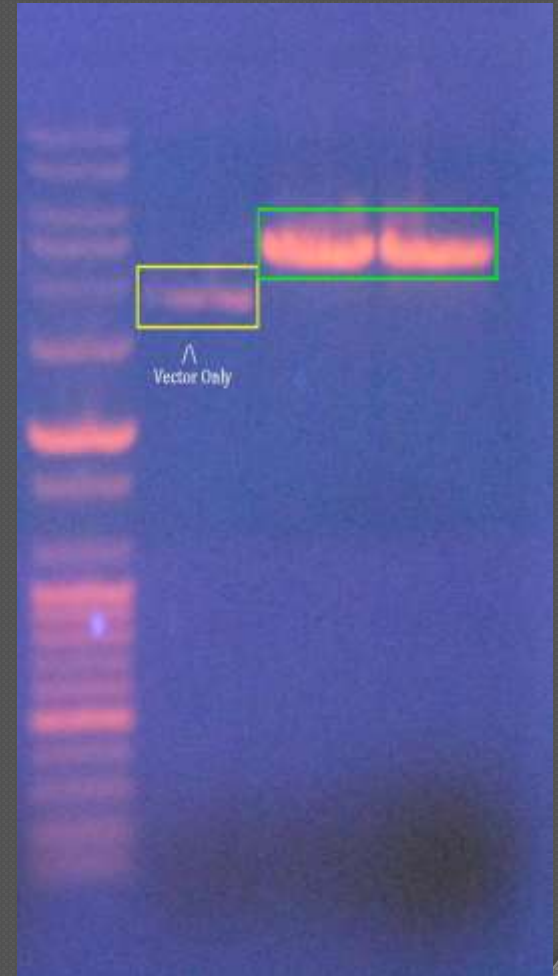
- *Salmonella enterica* serovar Newport
- Uncharacterized Integrons
 - 1.0, 1.2, and 1.8 kb



(Willford et al. 2007)

Previous Study

- 1.2 kb integron 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

Research Objectives

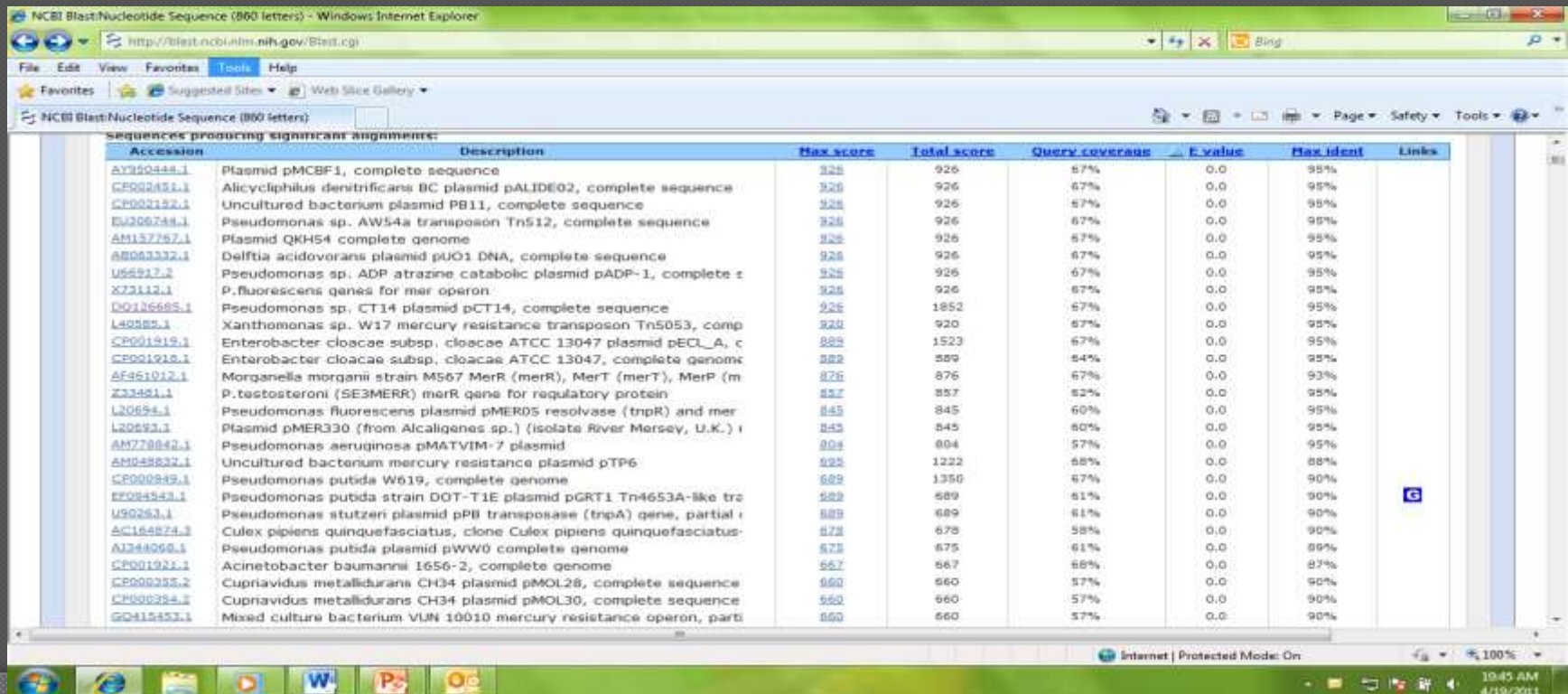
- Determine if trimethoprim resistance (1.2 kb) can be transferred between *Salmonella* strains, SN4 to PT30
- Characterize transferred genes for antibiotic resistance phenotypically and genotypically

Procedure

- SN4 (1.2 kb) and PT30 were grown individually
- Samples from each culture were then grown together in mixture, dilutions of the mixture were cultured on LB plates containing Naladixic Acid and Trimethoprim to select for PT30 containing the 1.2kb integron
- Transferred genes were sequenced

Results

- The 1.2 kb integron was not transferred from SN4 to PT30. A mercury resistance plasmid was transferred and sequenced.



NCBI Blast: Nucleotide Sequence (800 letters) - Windows Internet Explorer

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

NCBI Blast: Nucleotide Sequence (800 letters)

sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E-value	Max ident	Links
AY950444.1	Plasmid pMCBF1, complete sequence	926	926	87%	0.0	98%	
CP002451.1	Alicyclophilius denitrificans BC plasmid pALIDE02, complete sequence	926	926	67%	0.0	95%	
CP002152.1	Uncultured bacterium plasmid PB11, complete sequence	926	926	67%	0.0	95%	
EU206744.1	Pseudomonas sp. AW54a transposon Tn512, complete sequence	926	926	67%	0.0	95%	
AM157767.1	Plasmid QKH54 complete genome	926	926	67%	0.0	95%	
AB083332.1	Delftia acidovorans plasmid pUO1 DNA, complete sequence	926	926	67%	0.0	95%	
U56917.2	Pseudomonas sp. ADP atrazine catabolic plasmid pADP-1, complete s	926	926	67%	0.0	95%	
X72112.1	P. fluorescens genes for mer operon	926	926	67%	0.0	95%	
DQ136685.1	Pseudomonas sp. CT14 plasmid pCT14, complete sequence	926	1852	67%	0.0	95%	
L40885.1	Xanthomonas sp. W17 mercury resistance transposon Tn5053, comp	920	920	67%	0.0	95%	
CP001919.1	Enterobacter cloacae subsp. cloacae ATCC 13047 plasmid pECL_A, c	899	1523	67%	0.0	95%	
CP001918.1	Enterobacter cloacae subsp. cloacae ATCC 13047, complete genom	899	899	64%	0.0	95%	
AF461012.1	Morganella morganii strain M567 MerR (merR), MerT (merT), MerP (m	876	876	67%	0.0	93%	
Z33451.1	P. taetosteroni (SE3MERR) merR gene for regulatory protein	857	857	62%	0.0	95%	
L20694.1	Pseudomonas fluorescens plasmid pMERD5 resolvase (trpR) and mer	845	845	60%	0.0	95%	
L20693.1	Plasmid pMER330 (from Alcaligenes sp.) (isolate River Mersey, U.K.)	845	845	60%	0.0	95%	
AM778842.1	Pseudomonas aeruginosa pMATVIM-7 plasmid	804	804	67%	0.0	95%	
AM048827.1	Uncultured bacterium mercury resistance plasmid pTP6	822	1222	68%	0.0	88%	
CP000949.1	Pseudomonas putida W619, complete genome	689	1350	67%	0.0	90%	
EF084543.1	Pseudomonas putida strain DOT-T1E plasmid pGRT1 Tn4653A-like tra	689	689	61%	0.0	90%	G
U90261.1	Pseudomonas stutzeri plasmid pPB transposase (trpA) gene, partial	689	689	61%	0.0	90%	
AC164874.1	Culex pipiens quinquefasciatus, clone Culex pipiens quinquefasciatus-	678	678	58%	0.0	90%	
AJ244060.1	Pseudomonas putida plasmid pWW0 complete genome	673	675	61%	0.0	89%	
CP001921.1	Acinetobacter baumannii I656-2, complete genome	667	667	68%	0.0	87%	
CP000355.2	Cupriavidus metallidurans CH34 plasmid pMOL28, complete sequence	660	660	57%	0.0	90%	
CP000354.2	Cupriavidus metallidurans CH34 plasmid pMOL30, complete sequence	660	660	57%	0.0	90%	
GC415453.1	Mixed culture bacterium VUN 10010 mercury resistance operon, parti	660	660	57%	0.0	90%	

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Conclusions

- No proven transference of the 1.2kb integron gene cassette
- Identification of a mercury resistance gene cassette
 - Potential explanation for multiple resistance observed in SN strains

Future Work

- Characterize mercury resistance gene contribution to antibiotic resistance
- Cloning and 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
- Movement of integrons between *Salmonella* species

References

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Questions



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