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Renee S. Levings
University of Wollongong

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Detailed molecular analysis of antibiotic resistance
regions within a collection of multi-drug resistant
Salmonella spp. from Australian sources

A thesis submitted in fulfilment of the requirements for the award of the degree

Doctor of Philosophy

From

The University of Wollongong

By

Renee S. Levings

Department of Biological Sciences

2008

Thesis Declaration

I, Renee S. Levings, declare that this thesis is submitted in accordance with the regulations required of the University of Wollongong in fulfilment of the degree of Doctor of Philosophy, in the Department of Biological Sciences. This thesis does not include work previously published by another person unless appropriate reference is stated in the text. This document has not been submitted for qualifications at any other academic institution.

Renee S. Levings

May 2008

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Abstract

Salmonella spp., in particular *Salmonella* Typhimurium is an important zoonotic pathogen both here in Australia and internationally. Over the past few decades the use of antimicrobials in human, agricultural and aquacultural settings has created significant selection pressures, giving rise to multiply antibiotic resistant bacteria, including *Salmonella*. The acquisition and dissemination of the genes responsible for antimicrobial resistance has been largely attributed to mobile genetic elements, including Class 1 integrons and the gene cassettes they contain. The initial aim of this study was to examine a collection of 136 multiply resistant *Salmonella* of different serovars from varying Australian sources (predominately animal and to a lesser extent human) for the presence of Class 1 integrons and to identify the gene cassettes present. Using PCR to amplify up specific regions within the Class 1 integron structure, 51.4% of the isolates examined were found to contain the Class 1 integron associated *intI1* integrase. All of these, apart from 11 isolates, contained cassette arrays which were characterised using restriction enzyme analysis and DNA sequencing. The gene cassettes identified among the collection were almost solely responsible for resistance to trimethoprim and aminoglycosides. The *dfrA5* gene cassette (responsible for resistance to trimethoprim) was the most prevalent cassette, particularly among the bovine isolates. Three new gene cassettes responsible for resistance to aminoglycosides, trimethoprim and lincosamides (*aacCA5*, *dfrB6* and *linG*, respectively) were identified. SGI1 (*Salmonella* Genomic Island 1), a 43 kb chromosomal island known to contain a large multi-drug resistance integron, In104 was found to be present in 10 experimental isolates and associated with 4 new serovars, namely Kiambu, Dusseldorf, Cerro and Emek. The integron in the *Salmonella* Emek strain was found to have inserted via transposition at a unique site

within the island backbone and this structure has been named SGI2. This unique insertion site suggests that SGI2 has evolved independently of SGI1.

A second collection of multi-drug resistant *Salmonella* Paratyphi BdT⁺ isolates sourced from human infections in Melbourne and the corresponding home aquaria of infected patients were examined for clonality and the presence of SGI1. All *S. Paratyphi* BdT⁺ from infected individuals were indistinguishable from the isolates from their respective fish tanks, using IS200 profiling techniques and pulse field gel electrophoresis of *Xba*I digested chromosomal DNA. SGI1 (containing the *aadA2* and *blaP1* gene cassettes) was found to be present in all the *S. Paratyphi* BdT⁺ isolates examined. This is the first definitive molecular study showing that ornamental fish tanks are a reservoir for multiply resistant *Salmonella* Paratyphi BdT⁺. Studies examining the molecular mechanisms involved in antimicrobial resistance, and the way in which mobile elements are incorporated and clustered into large multi-drug resistance regions such as SGI1, provide useful information needed for the ongoing surveillance of multiply resistant *Salmonella* and other bacterial pathogens involved in outbreaks domestically and internationally.

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**Chapter 5: A molecular and epidemiological study of multiply
antibiotic resistant *Salmonella* Paratyphi BdT⁺ containing
SGI1 sourced from ornamental fish tanks and human
infections in Australia**

- 5.1** A summary of genetic characteristics of *Salmonella enterica* 112
serovar Paratyphi BdT⁺ isolates used in this study.

Abbreviations

aa	amino acid
ANGIS	Australian National Genomic Information Service
Ap	ampicillin
59 be	59 base element
bp	base pairs
CFU	colony forming units
Cm	chloramphenicol
CR	common region
5'CS	5' conserved segment
3'CS	3' conserved segment
Cp	ciprofloxacin
Cp'	intermediate resistance to ciprofloxacin
CSPD	Disodium 3-(4-methoxyspiro {1,2-dioxetane-3,2'-(5'-chloro) tricyclo [3.3.1.1 ^{3,7}] decan}-4-yl) phenyl phosphate
DANMAP	The Danish Integrated Antimicrobial Resistance Monitoring Program
dfr	dihydrofolate reductase
DHPS	dihydropteroic acid synthetase
DIG	digoxigenin
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleotide triphosphates
°C	degrees celsius
EDTA	ethylenediamine tetra-acetic acid
EMAI	Elizabeth Macarthur Agricultural Institute
Fl	florfenicol
<i>g</i>	<i>g</i> forces for centrifugation
<i>g</i>	gram
Gm	gentamicin
h	hour
HCl	hydrochloric acid
In	integron
Inc	Incompatibility group
IR	inverted repeats
IS	insertion sequence
JETACAR	Joint Expert Technical Advisory Committee on Antibiotic Resistance
kb	kilobases
kD	kilodalton
kg	kilogram
Km	kanamycin
kV	kilovolts
l	litre
LB	Luria Bertani
m	milli
M	molar
mm	millimetre
MDU	Microbiological Diagnostic Unit (Melbourne)
MIC	minimal inhibitory concentration
MMR's	multi-resistance regions
MR	multiply antibiotic resistant

μ	micro
min	minute
ml	millilitre
n	nano
Na	nalidixic acid
NaCl	sodium chloride
NaOH	sodium hydroxide
NCBI	National Centre for Biotechnology Information
NEPSS	National Enteric Pathogens Surveillance System
NNDSS	National Notifiable Diseases Surveillance System
OD	optical density
ORF	open reading frame
%	percentage
PBS	phosphate buffered saline
PCR	polymerase chain reaction
p	pico
pH	pondus Hydrogeni
PT	phage type
PFGE	pulse field gel electrophoresis
RDNC	results do not conform
RNA	ribonucleic acid
rpm	revolutions per minute
RT	room temperature
s	second
SDS	sodium dodecyl sulfate
SGI1	<i>Salmonella</i> Genomic Island 1
Sm	streptomycin
Sp	spectinomycin
spp.	species
SRC	<i>Salmonella</i> reference collection
SSC	sodium citrate
Su	sulphathiazole/sulfonamides
TBE	tris-borate EDTA
TE	tris EDTA
Tc	tetracycline
Tn	transposon
Tp	trimethoprim
Tra	transposition region
U	units
UV	ultra-violet
V	volts
v/v	volume/volume
w/v	weight/volume
WHO	World Health Organisation

Publications arising from this thesis

1. Levings, R. S., S. R. Partridge, S. P. Djordjevic, and R. M. Hall. (2007) SGI1-K, a variant of the SGI1 genomic island carrying a mercury resistance region, in *Salmonella enterica* serovar Kentucky. *Antimicrob Agents Chemother* **51**:317-23.
2. Levings, R. S., R. M. Hall, D. Lightfoot, and S. P. Djordjevic. (2006) *linG*, a new integron-associated gene cassette encoding a lincosamide nucleotidyltransferase. *Antimicrob Agents Chemother* **50**:3514-5.
3. Levings, R. S., D. Lightfoot, R. M. Hall, and S. P. Djordjevic. (2006) Aquariums as reservoirs for multidrug-resistant *Salmonella* Paratyphi B. *Emerg Infect Dis* **12**:507-10.
4. Levings, R. S., D. Lightfoot, L. D. Elbourne, S. P. Djordjevic, and R. M. Hall. (2006) New integron-associated gene cassette encoding a trimethoprim-resistant DfrB-type dihydrofolate reductase. *Antimicrob Agents Chemother* **50**:2863-5.
5. Levings, R. S., D. Lightfoot, S. R. Partridge, R. M. Hall, and S. P. Djordjevic. (2005) The genomic island SGI1, containing the multiple antibiotic resistance region of *Salmonella enterica* serovar Typhimurium DT104 or variants of it, is widely distributed in other *S. enterica* serovars. *J Bacteriol* **187**:4401-9.
6. Levings, R. S., S. R. Partridge, D. Lightfoot, R. M. Hall, and S. P. Djordjevic. (2005) New integron-associated gene cassette encoding a 3-N-aminoglycoside acetyltransferase. *Antimicrob Agents Chemother* **49**:1238-41.