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

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 in Doctor of Philosophy from The University of Wollongong.

By Renee S. Levings

Department of Biological Sciences

2008
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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<td>amino acid</td>
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<td>Ap</td>
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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 Salmonella Genomic Island 1
Sm streptomycin
Sp spectinomycin
spp. species
SRC Salmonella reference collection
SSC sodium citrate
Su sulphathiozole/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


