2009

Characterisation of antibiotic resistance gene clusters and their mobility within a collection of multi-drug resistant Salmonella spp

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University of Wollongong
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Characterisation of antibiotic resistance gene clusters and their mobility within a collection of multi-drug resistant Salmonella spp.

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

Doctor of Philosophy

from

University of Wollongong

by

Xiulan Liu

Department of biological sciences

2009
Declaration

I, Xiulan Liu, declare that the thesis submitted to the University of Wollongong in fulfillment of the degree of Doctor of Philosophy does not include any work published previously by another person unless appropriate reference is stated in the text. This material has not been submitted for qualifications at any other academic institution.

Signed: Xiulan Liu

Date: 25 May 2009
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List of Abbreviations

Ab = absorbance
Ap = ampicillin
APH = phosphotransferase
ARP = antibiotic resistance profile
attI = the gene cassette integration site
be = base element
bp = base pair
CFU = colony forming units
Claa = chloroform/isoamylalcohol
Cm = chloramphenicol
CR = common region
CS = conserved segment
Cp = ciprofloxacin
Cp' = intermediate resistance to ciprofloxacin
CSPD = disodium 3-(4-methoxyspiro {1,2-dioxetane-3,2′-(5′-chloro) tricycle [3.3.1.1^{3,7}] decan}-4-yl) phenyl phosphate
dfr = dihydrofolate reductase
DHPS = dihydropteroate synthase
dH2O = distilled water
DIG = digoxigenin
DNA = deoxyribonucleic acid
dNTPs = deoxynucleotide triphosphates
DT104 = definitive type 104
°C = degrees celsius
E. coli = Escherichia coli
EDTA = ethylenediaminetetraacetic acid
EMAI = Elizabeth Macarthur Agricultural Institute
ESBLs = extended spectrum beta lactamases
EU = European Union
NRA = National Registration Authority
Fl = florfenicol
FDA = the United States Food and Drug Administration
g = gram
Gm = gentamicin
GCK = gene construction kit
h = hour
HCl = hydrochloric acid
H2O = water
Hg = mercury
HgS = mercury sulphide
In = integron
Int = integrase
Inc = incompatibility
IR = inverted repeat
IS = insertion sequence
kbp = kilo base pairs
kg = kilogram
Km = kanamycin
KOH = potassium hydroxide
kV = kilo volt
L = litre
LB = Luria Bertani
M = molar
mA = milliamp
MgCl₂ = magnesium chloride
MDR = multidrug resistance
MDU = Microbiological Diagnostic Unit (Melbourne)
MIC = minimum inhibitory concentration
min = minute
ml = millilitre
mm = millimetre
MQ water = Milli-Q water
µ = micro
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
pH = potential of Hydrogen
PCIaa = phenol/chloroform/isoamylalcohol
PT = phage type
PFGE = pulsed-field gel electrophoresis
QAC = quaternary ammonium compound
QLD = Queensland
R plasmid = resistance plasmid
r-det = resistance determinant
RDNC = results do not conform
RNA = ribonucleic acid
rpm = revolutions per minute
RT = room temperature
RTF = resistance transfer factor
sec = second
SDS = sodium dodecyl sulfate
SGI1 = Salmonella Genomic Island 1
Sm = streptomycin
Sp = spectinomycin
spp. = species
SRC = Salmonella reference collection
SSC = sodium citrate
sul = sulfonamide
TBE = tris-borate-EDTA
Tc = tetracycline
TE = tris-EDTA
Tp = trimethoprim
tra = transposition
tRNA = transfer RNA
U = units
UV = ultraviolet
USA = United States of America
UK = United Kingdom of Great Britain and Northern Ireland
V = volt
v/v = volume/volume
w/v = weight/volume
WHO = World Health Organisation
Abstract

One hundred and thirty-six Salmonella enterica strains, isolated from humans, animals, environmental and plant sources in Australia from 23 serovars, were examined for the streptomycin resistance gene strA and strB, the sulfonamide resistance gene sul2, and the tetracycline resistance gene tetA(A) and tetA(B). Thirteen strains were identified as containing the strA-strB genes located on the transposon Tn5393. S. enterica serovar Hadar accounted for 11 of these strains, 6 of which were isolated from humans and 5 were from ducks. This investigation is therefore the first report of the Tn5393 transposon being detected in bacterial strains from a human source in Australia.

RSF1010 plasmids were identified and extracted from 4 S. enterica strains, and were further confirmed by restriction enzyme profiling using PstI, SspI and EcoRV. Small non-conjugative plasmid p9123 was extracted and characterised from 3 of the S. enterica strains and also confirmed by restriction enzyme digestion. An RSF1010-like plasmid was also identified in 3 of the strains. This plasmid was found to be approximately 2.6 kb larger than RSF1010, and possibly derived from the RSF1010 plasmid via insertion of the tetracycline resistance gene tetA(A) between strB and mobC genes.

An IS26-strB-strA-sul2-repC-repA-IS26 antibiotic resistance region was identified in 33 S. enterica strains, among these were 23 serovar Typhimurium isolates, 8 serovar
Bovismorbificans, 1 serovar Senftenberg and 1 isolate where the serovar could not be conclusively identified. The 23 Typhimurium strains were further characterised by PCR and Southern hybridisation analysis using a $bla_{TEM}$ gene probe. The analysis identified two classes of antibiotic resistance gene clusters. Eleven $S. enterica$ serovar Typhimurium strains harboured an IS26-$strB-strA-sul2-repC-repA-IS26-bla_{TEM-1}$-IS26 antibiotic resistance gene cluster and another 10 $S. enterica$ serovar Typhimurium strains contained an IS26-$strB-strA-sul2-repC-repA-IS26-bla_{TEM-1}$ gene cluster, without the IS26 element downstream of the $bla_{TEM-1}$ gene. Two strains contain elements of these gene clusters but further investigation is needed to fully identify these.

Further linkage PCR amplifications revealed that the IS26-$strB-strA-sul2-repC-repA-IS26-bla_{TEM-1}$-IS26 antibiotic resistance gene cluster was possibly inserted into the 3′-CS of a class 1 integron (In4 type) and truncated the 3′-CS region. Three derivatives were identified, of which the $dfrA5-intI1$ type was most commonly found downstream of the $bla_{TEM-1}$-IS26 region. Southern hybridisation analysis using an IS200 gene probe revealed that strains which contain different antibiotic resistance gene clusters also display different but related IS200 profiles.

The antibiotic resistance gene clusters of 19 $S. enterica$ serovar Typhimurium strains were transferred to an $E. coli$ 294 Rif$^r$ recipient either by direct mating or triparental mating methods. These experiments confirmed that the antibiotic resistance gene clusters were located on conjugative or mobilisable plasmids. The antibiotic resistance
gene clusters of 4 *S. enterica* serovar Typhimurium strains could not be transferred to the *E. coli* 294 Rif$^\text{r}$ recipient. These experimental results suggest that the antibiotic resistance gene cluster of IS26-strB-strA-sul2-repC-repA-IS26-bla$\text{TEM-1}$-IS26 might move as one genetic element between distinct plasmid backbones.
Acknowledgements

I would like to thank my supervisor Professor Mark Walker first. From the beginning, he trusted me and gave me the chance to start my PhD. In the next 5 years, he gave me great support with regards research direction. Especially during the writing of my thesis, he spent a lot of time exchanging ideas and discussing directions for my thesis. I would like to thank him for his generosity and tolerance in the past few years. I would also like to thank my other supervisor Dr Steven Djordjevic, who gave me much useful advice, set the direction for my research project and supported my thesis writing.

I would like to thank Dr Renee Levings, who acted as my "third supervisor" and also as a good friend. In the past 5 years, she provided me great support not only with regards research direction but also in technical support. I would also thank her for spending a lot of time to help me with my thesis. As a friend, Renee also gave me great support to help me culturally adapt to Australia.

I would like to thank Dr Cheryl Jenkins and Dr Tracey Kuit, who both provided great friendship and a lot of laughs in the lab.

I would like to thank Carola Venturini, who shared her experimental experiences with me unselfishly.

I would like to thank Linda Falconer for her technical support.

I would like to thank Ania and Daniel, who were fun to work with in the lab.

I would like to thank Fay Dawes for her primers.

I would like to thank my other colleagues at EMAI as well, who provided a friendly environment to work in.

Finally and most importantly, I would like to thank my husband Ying Jiang and my son Qi Jiang, for their love, understanding and tolerance of my absence from home for five years.
Publications Arising from this Thesis

Paper 1
Characterisation of resistance genes in multiply antibiotic resistant Salmonella enterica serovar Typhimurium from human and bovine sources
Renee S. Levings¹, Xiulan Liu¹,³, Diane Lightfoot⁴, Nick Evershed⁵, Linda Falconer¹, Mark J. Walker³, Ruth M. Hall⁵ and Steven P. Djordjevic¹,²*

Paper 2
Persistence of RSF1010-like plasmids and origin of their sul2-strA-strB antibiotic resistance gene cluster
Sheree Yau, Xiulan Liu, Steven P. Djordjevic and Ruth M. Hall

Manuscript 1
Evolution of the Tn1696 transposon family
Amy K. Cain, Xiulan Liu, Steven P. Djordjevic and Ruth M. Hall