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

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

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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
CIaa = 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
dH_2O = distilled water
DIG = digoxigenin
DNA = deoxyribonucleic acid
dNTPs = deoxynucleotide triphosphates
DT104 = definitive type 104
ºC = 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
H_2O = 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
$MgCl_2$ = 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
Tn = transposon
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 Tn*5393*. *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 Tn*5393* 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 *Pst*I, *Ssp*I and *Eco*RV. 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 IS*26*-*strB*-*strA*-*sul2*-*repC*-*repA*-IS*26* 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 IS*26*-*strB*-*strA*-*sul2*-*repC*-*repA*-IS*26*-*bla*$_{TEM-1}$-IS*26* antibiotic resistance gene cluster and another 10 *S. enterica* serovar Typhimurium strains contained an IS*26*-*strB*-*strA*-*sul2*-*repC*-*repA*-IS*26*-*bla*$_{TEM-1}$ gene cluster, without the IS*26* 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 IS*26*-*strB*-*strA*-*sul2*-*repC*-*repA*-IS*26*-*bla*$_{TEM-1}$-IS*26* 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*$_{TEM-1}$-IS*26* region. Southern hybridisation analysis using an IS*200* gene probe revealed that strains which contain different antibiotic resistance gene clusters also display different but related IS*200* 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^r recipient. These experimental results suggest that the antibiotic resistance gene cluster of IS*26*-*strB*-*strA*-*sul2*-*repC*-*repA*-IS*26*-bla_{TEM-1}-IS*26* might move as one genetic element between distinct plasmid backbones.

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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. Levings[1], Xiulan Liu[1,3], Diane Lightfoot[4], Nick Evershed[5], Linda Falconer[1], Mark J. Walker[3], Ruth M. Hall[5] and Steven P. Djordjevic[1,2*]

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