Temperate bacteriophages and the molecular epidemiology of antibiotic resistance in *Salmonella enterica*

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Thesis Summary

Foodborne diseases caused by non-typhoidal *Salmonella* represent an important public health problem worldwide (Zhao *et al.*, 2003). The transmission of *Salmonella* between animals and humans has been well established in epidemiological studies. In the case of complicated illness caused by *Salmonella* where antibiotics need to be administered, treatment can be compromised if the infecting organism is resistant to the prescribed antimicrobial agent. This study and earlier studies have shown that many *Salmonella* carry temperate bacteriophages as lysogens. Many of these bacteriophages are capable of mediating generalised transduction (Schicklmaier and Schmieger, 1995; Schicklmaier *et al.*, 1998; Mmolawa *et al.*, 2002). Schmieger and Schicklmaier (1999) demonstrated that bacteriophages ES18 and PDT17 are capable of transduction of antibiotic resistance genes from DT104.

Phage-mediated transduction of antibiotic resistance genes has been largely neglected in the study of genetic transfer of antibiotic resistance in bacteria. This study investigates whether bacteriophages exist in antibiotic resistant *Salmonella* isolates. Such temperate phages in antibiotic resistant isolates could play a significant role in the transfer of resistance to other species of enteric bacteria, such as *E. coli.*

Molecular epidemiology studies of antibiotic resistance genes were undertaken with *Salmonella* isolates from chicken, pig and human sources that were subjected to PCR for ampicillin ($bla_{\text{TEM-1}}$), tetracycline (*tet*A, *tet*B) and streptomycin (*aad*A1, *aad*A2, *str*A, *str*B) resistance genes as well as Class 1 integrons. The $bla_{\text{TEM-1}}$ gene was widely detected in isolates from pigs and chickens but rarely detected in human isolates. The *tet*B gene was more commonly found in pig isolates, while the *tet*A gene was associated with tetracycline resistance in chicken isolates. The *str*A and *str*B genes were responsible for streptomycin resistance in the *S.* Typhimurium isolates while the *aad*A1 gene was commonly detected in *S.* Kiambu and *S.* Virchow isolates. The *aad*A2 gene was associated with streptomycin resistance in the *S.* Ohio isolates from pigs. Class 1 integrons were widely distributed across serovars tested from chicken, pig and human sources.

Temperate bacteriophages were induced using mitomycin C from antibiotic resistant *Salmonella*. These phages were able to infect antibiotic-sensitive *Salmonella* isolates from humans. Bacteriophages induced from one *S.* Sofia isolate also plaqued on *Shigella flexneri.* Bacteriophages induced from one *S.*Kiambu isolate and *S.* Typhimurium DB21 with an inserted *Tn*10 transposon (*S.* Typhimurium DB21 *Tn*10) were capable of transducing ampicillin and tetracycline resistance, respectively into *S.* Enteritidis PT1 isolates by *in vitro* methods. The molecular basis for resistance was established in subsequent PCR for antibiotic resistance genes in donor and recipient strains. This finding, in particular in the wild-type *S.* Kiambu strain, indicates that *Salmonella* from a natural source are able to infect and transfer antibiotic resistance by generalised transduction in controlled laboratory experiments.

This current study has investigated the transfer of tetracycline and ampicillin resistance from a wild-type *Salmonella* strain and a laboratory strain of *Salmonella* to wild-type *Salmonella* bacteria as it occurs within the normal flora of the chicken gastrointestinal tract. It was demonstrated that the genetic transfer of tetracycline and ampicillin resistance genes as well as Class 1 integrons can occur within the chicken gastrointestinal tract. Transfer of tetracycline and ampicillin resistance could be demonstrated both *in vitro* and by using bacteriophage lysates obtained from *in vivo* studies in transduction experiments. It was clearly shown that bacteriophage isolated from chicken faeces and caeca could infect antibiotic sensitive recipient *Salmonella*. Interaction between phages of the administered *Salmonella* strains may be occuring with phages of bacteria in the normal flora allowing previously inactive phage in the indigenous flora to plaque on indicator strains.

Additionally, strong evidence was presented to suggest that the environment of the chicken gastrointestinal tract could mediate phage type conversion in recipient and transductant strains. Phage typing of these recipient and transductant strains demonstrated a trend for recipient strains to become more resistant to phages in the *S.* Enteritidis typing panel. This led to weakened phage reactions such RDNC (reaction does not conform) and untypable. The acquisition of phages may be a way for *Salmonella* to enhance competitive fitness and generate new strains in order to evolve and diversify. Or the acquisition of plasmids either by transduction or conjugation may also mediate phage type conversion.

MLVA typing was performed on selected recipient, donor and transductant strains. The changes to tandem repeat loci in *Salmonella* isolates that have passed through a chicken

gastrointestinal tract have not been described before. The changes to fragment length suggest that the bacterial chromosome is undergoing rearrangement; this may be attributed to a number of factors including acquisition of phages, prophage integration into tRNA sites, slipped-strand mispairing or the adaption to changing environment, in this case the chicken gastrointestinal tract.

This study has provided molecular epidemiological data on the antibiotic resistance genes and integrons present in Australian *Salmonella* isolates from human and animal sources. Information on the role of bacteriophages in the transfer of antibiotic resistance genes *in vitro* and in a chicken gastrointestinal tract has also been established.

List of Abbreviations

^{o}C	degrees Celsius
β	beta
AFLP	Amplified Fragment Length Polymorphism
AGP	animal growth promoter
Amp	ampicillin
BA	blood agar
BHI	brain heart infusion
bp, kb	base pair (s); kilobase(s)
cfu	colony forming units
CLSI	Clinical Laboratory Standards Institute
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
dsDNA	double stranded DNA
DT	Definitive type
EDTA	ethylene diamine tetra acetic acid
EMEA	The European Agency for the Evaluation of Medicinal Products
ESBL	Extended spectrum beta-lactamase
^{I}	intermediate resistance
JETACAR	Joint Expert Technical Advisory Committee on Antibiotic Resistance
L; mL, µL	litre (s); millilitre (s); microlitre(s)
LB	Luria-Bertani
M; mM; µM	moles per litre, millimoles per litre; micromoles per litre
MAPLT	Multiple Amplification of Phage Loci Typing

mD	mega Daltons
mg; µg	milligram (s); microgram (s)
MAPLT	Multiple Amplification of Phage Locus Typing
MH	Mueller-Hinton
MIC	minimum inhibitory concentration
MLEE	Multilocus Enzyme Electrophoresis
MLST	Multilocus Sequence Typing
MLVA	Multilocus Variable Number Tandem Repeat Analysis
MRSA	methicillin-resistant Staphylococcus aureus
nt	nucleotide
nM	nanometres
ORF	open reading frame
p	plasmid
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PT	Phage type
R	resistance
RDNC	reaction does not conform
RFLP	Restriction Fragment Length Polymorphism
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
rpm	revolutions per minute
RT-PCR	real time PCR
S	sensitive
SDS	sodium dodecyl sulphate

STTR	*Salmonella* Typhimurium Tandem Repeat
TBE	Tris-borate EDTA
Tet	tetracycline
Tn	transposon
U	unit (s)
UT	untypable
UV	ultraviolet
VNTR	variable number of tandem repeats
VRE	vancomycin resistant enterococci
w/v	weight per volume
XLD	Xylose-Lysine-Desoxycholate

Publications

Heuzenroeder, M. W., Barton, M. D., Davos, D. and Tan, S. (2007). Molecular epidemiology of antibiotic resistance in*Salmonella* from Chickens: Antibiotic resistant *Salmonella* in Chickens, A report for the Rural Industries Research and Development Corporation. Canberra, Australia, Australian Government, Rural Industries Research and Development Corporation.