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**MOLECULAR EPIDEMIOLOGY OF *SALMONELLA* TYPHIMURIUM DT160
IN NEW ZEALAND**

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Abstract

Salmonellosis is a zoonotic bacterial disease of national and international importance. In New Zealand (NZ), the most common foodborne notifiable disease is campylobacteriosis, which is followed by salmonellosis. In 1998, *Salmonella enterica* subsp. *enterica* serotype Typhimurium Definitive Type 160 (DT160) was identified in NZ. Since first reported, *S. Typhimurium* DT160 has caused several epidemics in the country but has not produced significant illness worldwide.

Therefore, the objectives of the project were to investigate the molecular epidemiology of *S. Typhimurium* DT160 and the association between isolates from human and animal origin. Ninety *Salmonella* isolates obtained in the period between 1999 and 2009 from the Institute of Environmental Science and Research, NZ were assessed for colony morphology, serotype, susceptibility to 11 antimicrobials, virulotyped using Polymerase Chain Reaction (PCR) and the Pulsed Field Gel Electrophoresis (PFGE) patterns were also determined. In addition, 4 isolates were further assessed with Triple Sugar Agar, API20E biochemical and motility tests.

All 90 isolates were confirmed as *Salmonella* spp. with no indications for resistance to multiple antimicrobials. All isolates were susceptible to the antimicrobials used in this study with the exception of 26 and 8 isolates that had intermediate susceptibility against tetracycline and oxytetracycline, respectively. In an attempt to discriminate between potentially pathogenic and pathogenic *Salmonella* isolates, PCR-based virulotyping was performed based on 12 potential virulence genes. Results revealed that all isolates were positive for at least 10 of the 12 virulence genes. Two of the six isolates negative for one of the virulence genes (*invA*, *iroN*, *pefA* or *sifA*) were of human origin and the remaining four were sparrow

isolates. The PFGE patterns determined with restriction enzymes *XbaI* and *SpeI* demonstrated that the genotype profile AA1 accounted for 78/90 (86.7%) of the isolates, whilst the second most common profile, AA2, was found in only three isolates (3.3%), comprising two isolates from sparrows and one from a human. The remaining nine profiles were found in single isolates. All isolates of AA2 profile were PCR negative for *sifA*.

In conclusion, no obvious correlation was observed between the phenol- and geno-type and the isolates, year and month of isolation, and source of the samples. There was no obvious evidence for multidrug resistance among DT160 isolates. The PFGE and virulotyping profiles suggest close relation among majority of isolates with predominant and epidemiologically important genotype persistent in multiple hosts. Finally, the few genotypes with low prevalence in multiple hosts may indicate emergence of sporadic genomic variants in the population.

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“So, verily, with every difficulty, there is relief. Verily, with every difficulty there is relief”

(HQ 94:5-6)

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List of Abbreviations

ACSSuT	AMPICILLIN, CHLORAMPHENICOL, STREPTOMYCIN, SULPHONAMIDES, TETRACYCLINE
AK	AMIKACIN
AMC	AMOXICILLIN-CLAVULANIC ACID
AMP	AMPICILLIN
ATCC	AMERICA TYPE CELL CULTURES
BD	BECTON DICKINSON
bp	BASE PAIRS
C	CHLORAMPHENICOL
CDC	CENTERS FOR DISEASE CONTROL AND PREVENTION
CIP	CIPROFLOXACIN
CLB	CELL LYSIS BUFFER
CLSI	CLINICAL AND LABORATORY STANDARDS INSTITUTE
CPD	CEFPODOXIME
CSB	CELL SUSPENSION BUFFER
DNA	DEOXYRIBONUCLEIC ACID
DT	DEFINITIVE PHAGE TYPE
EDTA	ETHYLENEDIAMINETETRAACETIC ACID
ESR	INSTITUTE OF ENVIRONMENTAL SCIENCE AND RESEARCH
EU	EUROPEAN UNION
FOX	CEFOXITIN
kbp	KILOBASE PAIRS
LPS	LIPOPOLYSACCHARIDE
MDR	MULTI DRUG RESISTANT
MgCl ₂	MAGNESIUM CHLORIDE
MIN	MINUTES
mL	MILLILITRES
MLVA	MULTILOCUS VARIABLE NUMBER TANDEM REPEAT ANALYSIS
mM	MILLIMOLAR
mm	MILLIMETERS
NA	NALIDIXIC ACID
NCTC	NATIONAL COLLECTION OF TYPE CULTURES
NTP	NUCLEOTIDE TRIPHOSPHATE
NZ	NEW ZEALAND
OT	OXYTETRACYCLINE
PCR	POLYMERASE CHAIN REACTION
PFGE	PULSED FIELD GEL ELECTROPHORESIS
PT	PHAGE TYPE
SKG	SEAKEM GOLD

SPI	<i>SALMONELLA</i> PATHOGENIC ISLAND
SXT	TRIMETHOPRIM-SULFAMETHOXAZOLE
TBE	TRIS-BORATE EDTA
TE	TRIS-EDTA
TET	TETRACYCLINE
TTSS	TYPE III SECRETION SYSTEM
TSI	TRIPLE SUGAR IRON
USA	UNITED STATE OF AMERICA
WHO	WORLD HEALTH ORGANISATION