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MOLECULAR EPIDEMIOLOGY OF *SALMONELLA* TYPHIMURIUM DT160 IN NEW ZEALAND

A thesis presented in partial fulfilment of the requirements for the degree of

MASTER OF VETERINARY STUDIES

IN

PUBLIC HEALTH

AT MASSEY UNIVERSITY, PALMERSTON NORTH,

NEW ZEALAND

SHARINA OMAR

2010

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 *Xba*I and *Spe*I 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.

Acknowledgements

The acknowledgement is the last part of my thesis that I have to write but it is definitely not the least important, as I would not have been able to accomplish this work without the help of my supervisors, family and friends.

It has been a great opportunity for me to have been able to further advance my post graduate education at Massey University. This experience has nourished me with knowledge and I have gained vast experience working with people from a number of different backgrounds.

First of all, I would like to express my deepest gratitude to the Vice-Chancellor of University Putra Malaysia, the Dean and Head of the Department of Pathology and Microbiology at the Faculty of Veterinary Medicine for having confidence in me to carry out this responsibility and supporting me during this process.

I am too much indebted to the Malaysian Government for funding my two years of education. Without this help, it would be impossible for me to pursue my scientific training.

My special thank you goes to my enthusiastic main supervisor, Dr. Daniel Petkov who has patiently provided me with knowledge in molecular biology by sharing his experiences in this field throughout the course of the study. Thank you for the guidance particularly on academic writing skills and giving me the chance to explore in details the demanding molecular techniques that I will need for a strong scientific career. It was a pleasure as well to work with my two co-supervisors, Prof. Nigel French and Dr. Julie Collin-Emerson who have given me the opportunity to work on this project. Their advice and assistance is much appreciated.

In addition to my supervisors, I am also grateful and lucky to have met Errol Kwan, Lynn Rogers, Rebecca Pattison, Rukhshana Akhter and Angie Reynolds who were always there for me when I needed excellent technical assistance. It was wonderful working with all of you! And also to Shoukai Yu and Hamid Irshad who have spent their precious time in assisting me in the Kappa analysis and the pulsed-field gels and PFGE analysis respectively.

Furthermore, I would like to acknowledge Dr. Phil Carter and Dr. Muriel Dufour from the Institute of Environmental Science and Research Limited, New Zealand (NZ) for supplying the *Salmonella* isolates used in this study. This work was supported by grant 11406 from the Ministry of Agriculture and Forestry Biosecurity, NZ.

To my family and friends, in New Zealand and overseas, Thank you so much for all the love, care and moral support and making my daily life fun all the way through. God bless.

"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	*SALMONELLA* 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