Genetic Diversity Of *Salmonella typhi*

By

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For my Amma and Acha,

who have made me what I am.

.......... To my sisters, brothers and Ernst-Theo for believing

in me and giving me their love, strength and support.
Statement

AFLP analysis of the *Salmonella typhi* isolates in Chapter 4 was performed by Dr. Edgar Schreiber, PE Biosystems Foster City, California, U. S. A.

The remainder of the work described in this thesis is my own original work. I carried out the SSCP analysis (Chapter 5) in the laboratory of Prof. Martin Altwegg, Department of Medical Microbiology, University of Zürich, Zürich, Switzerland.

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Abbreviations

°C  degree Celcius
%   percent
ng  nanogram
μg  microgram
mg  milligram
g   gram
nm  nanometer
mm  millimeter
μl  microlitre
ml  millilitre
l   litre
pmol picomole
μM  micromole
mM  millimolar
M   molar
sec second
min minute
hr  hour
bp  base pair
kb  kilobase pair
Mb  megabase pair
MD  megadalton
V   voltage
v   volume
v/v volume per unit volume
w/v weight per unit volume
U   units of enzyme
OD  optical density
UV  ultraviolet
rpm revolutions per minute
e.g. example
λ lambda
Fig. figure
DI discriminatory index
IS insertion sequence
LB Luria Bertani
F fatal
NF non-fatal
PNG Papua New Guinea
MMR methyl-directed mismatch repair
SPI Salmonella pathogenicity island
W.H.O. World Health Organization
DNA deoxyribonucleic acid
RNA ribonucleic acid
dATP deoxyadenine triphosphate
dCTP deoxycytosine triphosphate
dGTP deoxyguanidine triphosphate
dTTP deoxythymidine triphosphate
dNTPs deoxyribonucleoside 5' triphosphate
spp. species
subsp. subspecies
S. Salmonella
RE restriction endonuclease
RFLP restriction fragment length polymorphism
PT phage type
MLEE multilocus enzyme electrophoresis
PCR polymerase chain reaction
RAPD random amplified polymorphic DNA
rep repetitive extragenic palindromic sequences
AFLP amplified fragment length polymorphism
SSCP single strand conformation polymorphism
PFGE pulsed-field gel electrophoresis
Abstract

Typhoid fever is a unique human septemic infection caused by *Salmonella typhi*. Typhoid fever is still an important public health problem in many developing countries. The continuing presence of the disease in endemic areas and emergence of multidrug-resistant strains in many developing countries as well as the increasing reports from developed countries has renewed the interest to better understand the epidemiology of typhoid fever and some aspects of its pathogenesis. Characterization of *S. typhi* strains in different epidemiological settings depends largely on the utility of highly precise molecular typing tools.

A convenient, versatile and safe method for preparing bacterial DNA for ribotyping, pulsed-field gel electrophoresis (PFGE), IS200 typing and gene hybridization of restricted DNA analysis has been described in this study.

PFGE analysis, IS200 typing, ribotyping, amplified fragment length polymorphism (AFLP) analysis and gene hybridization profile typing clearly demonstrated the high genetic diversity among geographical isolates of *S. typhi*, indicating the existence of multiple clones of *S. typhi* in different regions of the world. On the other hand, limited diversity was observed among *S. typhi* strains isolated from patients in Papua New Guinea (PNG) with fatal and non-fatal typhoid fever by the same typing methods. This points to the fact that *S. typhi* strains circulating in PNG were possibly derived from single or closely related clones.

PCR-ribotyping and PCR-restriction fragment length polymorphism (RFLP) were found to be of limited value in subtyping *S. typhi*.

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