Genetic Diversity Of Salmonella typhi

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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*.

AFLP analysis of the PNG *S. typhi* strains from fatal and non- fatal cases of typhoid fever showed no association between genotypes (molecular profiles) and virulence (the

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