MOLECULAR CHARACTERIZATION OF MULTIDRUG-RESISTANT *Salmonella* Isangi IN HOSPITALIZED PATIENTS IN SOUTH AFRICA

Tersia Kruger

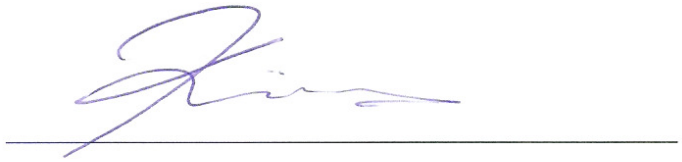
A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree Master of Science in Medicine

2007

Johannesburg

DECLARATION

I, Tersia Kruger, declare that this dissertation is my own work. It is being submitted for the degree of Master of Science in Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.



Signature of candidate

13th day of JUNE, 2007

PUBLICATION

Kruger, T., Szabo, D., Keddy, K.H., Deeley, K., Marsh, J.W., Hujer, A.M., Bonomo, R.A., Paterson, D.L. 2004. Infections with non-typhoidal *Salmonella s*pecies producing TEM-63 or a novel TEM Enzyme, TEM-131, in South Africa. Antimicrobial Agents and Chemotherapy **48**:4263-4270. (Appendix Four)

PRESENTATIONS

Kruger, T., Keddy, K.H., Sooka, A. Incidence of an extended-spectrum beta-lactamase producing *Salmonella* Isangi in South Africa. Presented at the Joint Congress: HIV Clinicians, Infectious Diseases, Infection Control, Travel Medicine, Sexually Transmitted Diseases Societies and Veterinary and Human Public Health, Stellenbosch, Cape Town, South Africa, 2-6 December 2001.

Wadula, J., Von Gottberg, A., Kilner, D., **Kruger, T.**, Sooka, A., de Jong, G., Cohen, C., Meyers, T., Khoosal, M., Keddy, K.H., Crewe-Brown, H. A Nosocomial outbreak in Pediatric Wards of *Salmonella* Isangi producing extended-spectrum beta-lactamases. Presented at the Joint Congress: HIV Clinicians, Infectious Diseases, Infection Control, Travel Medicine, Sexually Transmitted Diseases Societies and Veterinary and Human Public Health, Stellenbosch, Cape Town, South Africa, 2-6 December 2001.

Kruger, T., Keddy, K.H., Sooka, A. Incidence of an extended-spectrum beta-lactamase producing *Salmonella* Isangi in South Africa: An update, March 2000 – February 2002. Presented at the International Conference on Emerging Infectious Diseases (ICEID), Atlanta, Georgia, USA, 24-27 March 2002.

Kruger, T., Keddy, K.H. Evaluation of the ESBL Etest to identify extended spectrum beta-lactamase production in a nosocomial outbreak of *Salmonella* serotype Isangi. Presented at the 23 rd International Congress of Chemotherapy, Durban, South Africa, 7-9 June 2003.

ABSTRACT

Extended-spectrum beta-lactamase (ESBL)-producing *Salmonella enterica* serotype Isangi has emerged as a common *Salmonella* serotype affecting mainly children in hospitals throughout South Africa. Between 2000 and 2002, 279 *S*. Isangi isolates from single infection episodes were referred from 21 hospitals in 5 provinces to the Enteric Diseases Reference Unit of the National Institute for Communicable Diseases of South Africa. All isolates were subjected to antibiotic susceptibility testing and three disk-diffusion methods confirmed ESBL-production in 273 isolates. PCR and nucleotide sequencing of 101 isolates identified TEM-1 (2%), TEM-63 (91%), a novel TEM-131 (7%), and SHV-5 (2%), but CTX-M was not found. Plasmid profiling produced types with 1 to 6 plasmids, 7.4kb to 166kb in size, which were neither serotype nor ESBL-type specific. Pulsed-field gel electrophoresis revealed four major clusters while sub-clusters with identical, or near identical banding patterns suggested extensive intra-hospital transmission and clonal spread between hospitals and provinces in South Africa.

ACKNOWLEDGEMENTS

All praise to God, who inspired me with will and courage, through the angels of friends and family to believe in myself and have faith and determination; despite the obstacles I had to endure in the last two years.

I would like to gratefully acknowledge my supervisor Professor Hendrik Koornhof for his insight, expert advice, guidance, gentle encouragement, as well as courage to take on this task at a fairly late stage and despite being extremely busy, always made time to engage in interesting discussions. It is an honour. Thank you to my co-supervisor, Dr. Anthony Smith for his technical advice and review of the three molecular chapters and Dr. Karen Keddy for her support during the initiation of this project.

The biggest thanks to my colleagues and friends: Sandrama Nadan, Chantel le Roux, Lorraine Arntzen, Leigh Dini, Dr. Jenny Rossouw, Irma Latsky, Debbie van der Sandt and Dr. Nicola Page for putting up with me. Thank you to Sandrama and Jenny for your technical help and advice. I am indebted to each and every one of you for your understanding, support, endless patience and encouragement when it was most needed.

I am also grateful to the following laboratories and individuals for their contributions:
A very special and huge thank-you to Dr. David Paterson and the staff of the Division of Infectious Diseases at the University of Pittsburgh, with special thanks to my kind and patient friend Dr. Dora Szabó. Thank you for sharing your valuable technical knowledge in a 6-week crash course that enabled me to complete the experimental work in this dissertation;
The Respiratory and Meningeal Pathogens Research Unit (RMPRU) for the sharing of laboratory equipment, especially Dr. Mignon du Plessis for her technical advice;
The Vector Control Reference Unit (VCRU) for welcoming me into their "home" for a while. Thank you for your enthusiasm, support and willingness to help;
The Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA), the coordinators and all laboratory and hospital staff who contribute to the national and "enhanced" surveillance program in South Africa;
Dr. Nelesh Govender, pathologist for The National Microbiology Surveillance Unit (NMSU) of the NICD, for the updated clinical information on *S*. Isangi in South Africa for 2005;
Greg Duncan-Traill and Tony Chaplin at Davies Diagnostics (PTY Ltd) for providing the ESBL Etests®;
The National Health Laboratory Service (NHLS) for the 3-year grant.
Dr. Linda Meyer from the University of Pretoria for the *E. coli* (CTX-M-14) strain;

To my parents, especially my mother, who has always supported my dreams and aspirations. Thank you for all that you are and all you have done for me.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

NOMENCLATURE

°C	degrees Celcius
A	adenine
aa	amino acid
Ag	antigen
AIDS	acquired immune deficiency syndrome
APA	amino-penicillanic acid
ATCC	American Type Culture Collection
AUG	clavulanic acid
β	beta
bp	base pair
BSAC	British Society for Antimicrobial Chemotherapy
C	cytosine
CAZ	ceftazidime
CARL	Carletonville Hospital
CHB	Chris Hani Baragwanath Hospital
CIP	ciprofloxacin
CLSI	Clinical Laboratory Standards Institute
CPD	cefpodoxime
CT	cefotaxime Etest
CTL	cefotaxime + clavulanic acid Etest
CTX	cefotaxime
DDD	double disk diffusion
DNA	deoxyribonucleic acid
dNTPs	deoxynucleoside triphosphate
DRC	Democratic Republic of Congo
EC	Eastern Cape Province
EDRU	Enteric diseases reference unit
EDTA	ethylenediaminetetraacetic acid
ESBL(s)	extended-spectrum beta-lactamase(s)
ESC	extended-spectrum cephalosporins
et al.	and others
EtBr	ethidium bromide
FEP	cefepime
FS	Free State Province
g	gram
G	guanine

GA	Gauteng Province
GERMS-SA	Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa
GM	gentamicin
GRAHAM	Grahamstown Hospital
GRNPT	Green Point Hospital
GSH	Grootte Schuur Hospital
h	hour
HIV	Human Immunodeficiency Virus
HJ	Helen Joseph Hospital
HPA	Health Protection Agency
IMI	imipenem
kb	kilobase
KZN	KwaZulu Natal Province
L	liters
LB	Luria-Bertani broth
LIV	Livingstone Hospital
MC-207,110	Phe-Arg-napthylamide dihydrochloride
MDa	megaDalton
MDR	multiple-drug resistance
$MgCl_2$	magnesium chloride
mg/ml	milligrams per milliliter
MIC	minimum inhibitory concentration
MILP	Milpark Hospital
min	minute
ml	milliliter
MLST	multi-locus sequence typing
MLVA	multi-locus variable nucleotide tandem repeat analysis
mM	millimolar
MP	Mpumulanga Province
MW	molecular weight
NA	nalidixic acid
N/A	not applicable
NaCl	sodium chloride
NCCLS	National Committee on Clinical Laboratory Standards
NCTC	National Collection of Type Cultures
ND	non-determinable
NHLS	National Health Laboratory Service

NICD	National Institute of Communicable Diseases
NJH	Johannesburg General Hospital
NMSU	National Microbiology Surveillance Unit
NP	Northern Province
nt	nucleotide
NT	not tested
NTS	non-typhoidal *Salmonella*
NW	North West Province
OXA	oxacillin
PBP(s)	penicillin-binding protein(s)
PCR	polymerase chain reaction
PC	Pathcare, Cape Town
PE	Port Elizabeth Hospital
PFGE	pulsed-field gel electrophoresis
pH	percentage hydrogen
PI	Pathogenicity island
PIE	Pietersburg Hospital
PML	cefepime + clavulanic acid Etest
RED	Red Cross Childrens Hospital
RNA	ribonucleic acid
RNase	ribonuclease
rmp	revolutions per minute
RSA	Republic of South Africa
RT	room temperature
RUS	Rustenburg Hospital
SDS	sodium-dodecyl sulphate
SHV	Sulfhydryl Variable
SPI	*Salmonella* pathogenicity island
ss	single stranded
SOU	South Rand Hospital
T	thymine
TAE	Tris-acetate-EDTA buffer
TAMB	Tambo Memorial Hospital
TBE	Tris-borate-EDTA buffer
TDA	Tryptophane-deaminase
TE	Tris-EDTA buffer
TEM	Temoniera

TEMB	Tembisa Hospital
TEMED	N,N,N',N'-tetramethyl-ethylenediamine
TET	tetracycline
Tris	Tris-(hydroxymethyl)-aminomethane
TSE	Tsepong Hospital
µg/ml	micrograms per milliliter
µl	microliters
U	uracil
UPGMA	unweighted pair group method with arithmetic averages
URE	urea
USA	United States of America
UV	ultraviolet
VNTR	variable number tandem repeat analysis
VP	Voges Proskauer
v/v	volume per volume
WC	Western Cape Province
WHO	World Health Organization
w/v	weight per volume
XP	pulsed-field gel electrophoresis banding pattern