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

Tersia Kruger

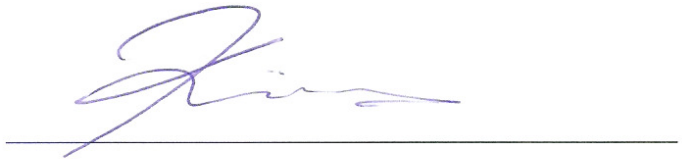
A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree Master of Science in Medicine

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.

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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