THESIS

MOLECULAR CHARACTERIZATION OF CARBAPENEMASE PRODUCING ENTEROBACTERIA (CPE) ISOLATED FROM A TERTIARY CARE TEACHING HOSPITAL IN SRI LANKA AND VALIDATION OF A RAPID CPE DETECTION PROTOCOL

Submitted by
MS. W.G.M. Kumudunie [B.Sc. (Hons)]
(FGS/05/MPhil/02/2017/01)

A thesis submitted to the Faculty of Graduate Studies, University of Kelaniya
in fulfillment of the requirements for the degree of
Master of Philosophy in Biochemistry

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DECLARATION

I declare that the work embodied in the thesis is my own and has not been submitted for any degree in this university or any other institute, and to the best of my knowledge and belief, it does not contain any material previously published or written or orally communicated by another person except, where due reference is made in the text.

.... Signature of the candidate

.... Date

To the best of our knowledge we endorse the declaration by the candidate.

Supervisors

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V. LIST OF ABBREVIATIONS

ABST	Antibiotic Sensitivity Testing
BA	Blood Agar
BHT	Bed Head Tickets
CDC	Centers for Disease Control and Prevention
CLSI	Clinical and laboratory Standards Institute
CNPt	Carba NP test
CNPt-std	Carba NP test – standard method
CNPt-direct	Carba NP test – direct method
CoV	Coronavirus
CPE	carbapenemase-producing enterobacteria
CRE	carbapenem-resistant *Enterobacteriaceae*
DDST	Double Disc Synergy Test
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleoside triphosphates
ESBL	Extended-spectrum beta-lactamase
ESBL-PE	Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GIM	German Imipenemase
GES	Guiana extended-spectrum
ICU	Intensive Care Unit
IMP	Imipenem hydrolysing beta-lactamase

IMI/NMC-A	Imipenemase/ non-metallo carbapenemase-A
KIA	Kligler Iron Agar
KPC	*Klebsiella pneumoniae* carbapenemase
LF	Lactose Fermentation
LRTI	lower respiratory tract infection
MALDI–TOF	matrix-assisted laser desorption ionization-time of flight mass
MS	spectrometry
MacA	MacConkey Agar
mCIM	modified carbapenem inactivation method
MDR	Multidrug Resistance
MDRE	Multidrug resistant *Enterobacteriaceae*
MDRO	Multidrug-resistant organisms
MERS	Middle East Respiratory Syndrome
MHA	Muller Hinton Agar
MHB	Muller Hinton broth
MHT	modified Hodge test
MIC	minimum inhibitory concentration
MRSA	methicillin-resistant *Staphylococcus aureus*
NDM	New Delhi metallo-beta-lactamase
Non-RE	non-resistant *Enterobacteriaceae*
OXA	Oxacillinase
PBP	penicillin-binding protein
PCR	polymerase chain reaction

SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
SFC-1	*Serratia fonticola* carbapenemase
SIM	Seoul Imipenemase
SLCM	Sri Lanka College of Microbiologists
SME	*Serratia marcescens* enzyme
SOP	standard operating procedure
TBE	Tris–borate EDTA
TSB	Tryptic Soy Broth
URTI	upper respiratory tract infection
USA	United States of America
UTI	urinary tract infection
VIM	Verona integron-encoded metallo-beta-lactamase
VRSA	Vancomycin-resistant *Staphylococcus aureus*
WBC	white blood cell
WHO	World Health Organization

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VII. ABSTRACT

Introduction: The emergence and spread of carbapenem-resistant *Enterobacteriaceae* (CRE) is in dramatic increase, resulting in failure of almost all the available antibiotics and hence limit the effective therapeutic options. Therefore, accurate and timely detection of carbapenemase-producing enterobacteria (CPE) is essential to streamline the optimum antibiotic therapy. This study was carried out to determine the current status of CRE in Sri Lanka and to evaluate the performances of CPE detection methods.

Methodology: A cross-sectional study was conducted at Colombo North Teaching Hospital during 2017-2018. Extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-PE) and CRE were identified by the disc diffusion method. CRE isolates were identified up to species level using a rapid identification kit. Four CPE detection methods, namely Carba NP test (CNPt), CNPt-direct, modified carbapenem inhibition method (mCIM), and modified hodge test (MHT) were evaluated. The genetic background of CPE was determined by PCR.

Results: The estimated overall prevalence of ESBL-PE and CRE were found to be 26.0% and 9.6%, respectively. The highest prevalence of ESBL-PE and CRE were found amongst uropathogenic (30.8%) and respiratory infections producing (20.8%) *Enterobacteriaceae*, respectively. *K. pneumoniae* (80.7%), *E. coli* (5.3%), *C. freundii* (7.0%), *P. rettgeri* (3.5%), *E. cloacae* (1.7%), and *E. aerogenes* (1.7%) were identified in CRE cohort. Of CRE, 94.7% were found to be CPE. The carbapenemase encoding genes detected were of bla_{KPC}, bla_{NDM}, and $bla_{OXA\text{-}48\text{-}like}$ and, $bla_{OXA\text{-}48\text{-}like}$ (88.9%) was the most prevalent. The overall sensitivity and specificity of CPE detection tests were as; MHT-90.7%, 92.1%, mCIM-100%, 100%, CNPt-75.9%, 100%, and CNPt-direct-83.3%, 100%, respectively. Only amikacin showed reasonable sensitivity (>50%) for CRE among the routine antibiotic panel whereas a higher level of susceptibility was noted for fosfomycin (92.9%), ceftazidime-avibactam (85.9%), and colistin (92.9%).

Conclusion: *K. pneumoniae* was the most prevalent CRE species. Carbapenemases production was the major resistance mechanism in CRE and $bla_{OXA\text{-}48\text{-}like}$ was the most prevalent gene type. The first occurrence of bla_{KPC} was recognized in Sri Lanka. MCIM and MHT had higher sensitivity compared to both CNP tests for the detection of CPE. However, when a prompt decision is needed, CNP tests can be a viable option since their results can be obtained within two hours.

Keywords – *Enterobacteriaceae*, ESBL, carbapenem resistance, carbapenemase, CNPt