MULTIDRUG RESISTANT *VIBRIO CHOLERAE* FROM DIARRHEAL STOOL SAMPLES OF NEPALGUNJ OUTBREAK AND DIFFERENT HOSPITALS OF NEPAL

A

DISSERTATION

SUBMITTED TO THE CENTRAL DEPARTMENT OF

MICROBIOLOGY

TRIBHUVAN UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR

THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE IN MICROBIOLOGY

(MEDICAL)

BY

EAK DEV KHANAL

CENTRAL DEPARTMENT OF MICROBIOLOGY

TRIBHUVAN UNIVERSITY

KIRTIPUR, KATHMANDU

NEPAL

2011

MULTIDRUG RESISTANT *VIBRIO CHOLERAE* FROM DIARRHEAL STOOL SAMPLES OF NEPALGUNJ OUTBREAK AND DIFFERENT HOSPITALS OF NEPAL

EAK DEV KHANAL

2011

RECOMMENDATION

This is to certify that Mr. Eak Dev Khanal has completed this dissertation work entitled **"Multidrug resistant *Vibrio cholerae* from diarrheal stool samples of Nepalgunj outbreak and different hospitals of Nepal"** as a partial fulfillment of Master of Science degree in Microbiology under our supervision. This dissertation work done by Mr. Khanal is an original research and has not been submitted to any other institute/Universities to earn any other degree.

…….	……..	……..
Dr. Dwij Raj Bhatta, Ph.D.	**Dr. Geeta Shakya, M.D.**	**Mr. Bishnu Prasad Upadhyay**
Head of Department	Acting Director	Senior Medical Technologist
Central Department of Microbiology	National Public Health Laboratory	NPHL, Teku
Tribhuvan University	Ministry of Health	
	Teku, Kathmandu	

Date: ……

CERTIFICATE OF APPROVAL

On the recommendation of **Dr. Dwij Raj Bhatta, Dr. Geeta Shakya and Mr. Bishnu Prasad Upadhyay**, this dissertation work of **Mr. Eak Dev Khanal** entitled as **"Multidrug resistant *Vibrio cholerae* from diarrheal stool samples of Nepalgunj outbreak and different hospitals of Nepal"** has been approved for the examination and submitted to the Tribhuvan University in the partial fulfillment for Master of Science degree in Microbiology (Medical).

…….

Dr. Dwij Raj Bhatta, Ph.D.

Head of Department

Central Department of Microbiology

Tribhuvan University

Kirtipur Kathmandu

Nepal

Date: ……..

BOARD OF EXAMINERS

Recommended by

……..

Dr. Dwij Raj Bhatta, Ph.D.

Supervisor

…….

Dr. Geeta Shakya, M.D.

Supervisor

…….....

Mr. Bishnu Prasad Upadhyay

Supervisor

Approved by ……

Dr. Dwij Raj Bhatta, Ph.D.

Head of Department

Examined by

…….....

Prof. Dr. Bharat Mani Pokhrel, Ph.D.

External examiner

…….....

Dr. Megha Raj Banjara, Ph.D.

Internal examiner

Date: ……

ACKNOWLEDGEMENT

I would like to extend my sincere and heartfelt gratitude to my supervisor Dr. Geeta Shakya and Mr. Bishnu Prasad Upadhyay for their constructive advice and supervision during the study period. I express my profound indebtness to them for providing the platform for my research work. A great appreciation of generosity goes to my supervisor Dr. Dwij Raj Bhatta for his guidance, constant and untiring inspiration, motivation and remarkable cooperation rendered to me during this research work.

I would like to share my eternal respect to all teachers of Central Department of Microbiology for their inspiration and support and encouragement. I specially thank lecturer of Central Department of Microbiology (CDM) Mr. Dev Raj Joshi for his warm advice and support during the laboratory work. I equally acknowledge the staffs of CDM and Special thank to Mr. Rayman Shakya for his valuable support during the study period.

My whole hearted thanks go to staffs of NPHL Mrs. Srijana Shrestha, Ms. Sailaja Adhikari and Mrs. Supriya Sharma for their support during the study period.

I am greatly thankful to my colleagues Ms. Goma Upadhyay, Mr. Kedar Bhaila Mr. Manoj Bhandari and Mr. Roshan Dahal for their effortful cooperation during the course of my study. Thanks to all those staffs and management committees of the different hospitals who provided me the samples for study. Special thanks to those volunteers who brought the outbreak samples to the laboratory.

But last not the least, my deepest and dearest admiration goes to all my family members for their tireless and unconditional support, continuous encouragement and blessings throughout my life.

Date: ……

Eak Dev Khanal

ABSTRACT

A nine month study was undertaken with an objective to isolate the multidrug resistant *Vibrio cholerae* from the diarrheal stool samples of Nepalgunj outbreak and different hospitals of Nepal. The laboratory work was conducted in National Public Health Laboratory Teku, Kathmandu. A total of 240 samples were processed in which 44 were from outbreak area and 196 were from hospitals. 48 cases were positive for cholera. The cholera agent was identified by biochemical test, serological test and biotyping methods which revealed that all strains of *Vibrio cholerae* were belong to serogroup O1 biotype El Tor and serotype Ogawa. Antibiotic susceptibility testing by disc diffusion method for 13 different antibiotics showed that 100% resistance to Nalidixic acid, Cotrimoxazole and Furazolidone, 100% sensitive to Amikacin Gentamicin, Ceftriaxone and Cefotaxime. 56.4% strains were resistant to Ciprofloxacin and 60.4% were resistant to Ofloxacin, 41.7% were sensitive to Chloramphenicol and Erythromycin. Minimum Inhibitory Concentration (MIC) test revealed that all the strains resistant during Antibiotic Susceptibility Test (AST) by disc diffusion method for Tetracycline, Nalidixic acid and Ampicillin were resistant. Only 4.2% out of 54.2% resistant strains detected from disc diffusion test were detected resistant in MIC test and other were intermediate for Ciprofloxacin. For Chloramphenicol none of the strains were detected resistant after MIC test. The mean MIC value for Ampicillin was 15.17 mg/L, Ciprofloxacin was 0.28 mg/L, Chloramphenicol was 1.42 mg/L, Nalidixic acid was 42.67 mg/L and Tetracycline was 4.16 mg/L. All Tetracycline resistant cases were detected from outbreak samples. 13 different R-type strains were detected after antibiogram testing. Effective monitoring and measurement of MIC level and further evaluation of the strains by molecular testing are needed.

Key words: Cholera, outbreak, AST, MIC, Ogawa, El Tor, *Vibrio*

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AC	-	Adenylate Cyclase
ADP	-	Adenosine Di-Phosphate
ADPR	-	ADP Ribose
APW	-	Alkaline Peptone Water
ASM	-	American Society of Microbiology
AST	-	Antibiotic Susceptibility Test
ATCC	-	American Type Culture Collection
ATP	-	Adenosine Tri-Phosphate
cAMP	-	cyclic Adenylate Mono-Phosphate
CBS	-	Central Bureau of Statistics
CDC	-	Center for Disease Control
CLSI	-	Clinical Laboratory Standard Institute
CT	-	Cholera Toxin
CTB	-	Cholera Toxin B
D	-	Dalton
DNA	-	Deoxy-Ribonucleic Acid
EDCD	-	Epidemic Disease Control Division
EHA	-	Emergency and Humanitarian Action
ETEC	-	Entero Toxigenic *E. coli*
EUCAST	-	European Committee of Antibiotic Sensitivity Testing
FDA	-	Food and Drug Administration
GTP	-	Guanosine Tri-Phosphate
ICE	-	Integrative and Conjugative Element

LPS	-	Lipopolysaccharide
MA	-	MacConkey Agar
MDR	-	Multidrug Resistant
MHA	-	Mueller Hinton Agar
MIC	-	Minimum Inhibitory Concentration
MR	-	Methyl Red
NA	-	Nutrient Agar
NCTC	-	National Culture Type Collection
NPHL	-	National Public Health Laboratory
PCR	-	Polymerase Chain Reaction
RBC	-	Red Blood Cells
RNA	-	Ribonucleic Acid
RND	-	Resistance Nodulation Division
SIM	-	Sulfide Indole Motility
SXT	-	Sulfomethoxazole
TCBS	-	Thio Sulfate Citrate Bile Sucrose Agar
TMP-SXT	-	Trimethoprim-Sulfomethoxazole
TSI	-	Triple Sugar Iron
TTGA	-	Tellurite Taurocholate Gelatin agar
VBNC	-	Viable But Non Culturable
VP	-	Voges Proskauer
WHO	-	World Health Organization

LIST OF TABLES

LIST OF FIGURE

LIST OF PHOTOGRAPHS

LIST OF APPENDICES