

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

A

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BY

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TRIBHUVAN UNIVERSITY
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SAMPLES OF NEPALGUNJ OUTBREAK AND DIFFERENT HOSPITALS OF NEPAL**

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2011

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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.

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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*

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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	-	Enterotoxigenic <i>E. coli</i>
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

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