

CONTENTS

	Page
ACKNOWLEDGEMENTS	i
ABSTRACT	ii
CONTENTS	v
LIST OF TABLES	viii
LIST OF FIGURES	x
LIST OF ABBREVIATIONS	xii
CHAPTER	
I INTRODUCTION	1
II SPECIFIC OBJECTIVE	3
III LITERRATURE REVIEW	4
1. <i>Vibrionaceae</i>	4
1.1 Clinical significance	4
2. <i>Vibrio cholerae</i>	6
2.1 Bacteriological characteristics	6
2.2 Laboratory diagnosis	7
2.2.1 Specimen collection	7
2.2.2 Microscopic examination	7
2.2.3 Culture method and biochemical identification	8
2.2.4 Serological characteristics	11
2.2.5 Immunodiagnosis using polyclonal antibodies	12
2.2.6 Immunodiagnosis using monoclonal antibodies	13
2.2.7 Polymerase chain reaction (PCR)	17
2.3 Epidemiology	22
2.3.1 Early pandemics	22
2.3.2 Seventh pandemic	23
2.3.3 Eighth pandemic	24
2.4 Molecular characteristic of <i>V. cholerae</i>	25
2.5 The molecular aspects of <i>V. cholerae</i> infections	27

CONTENTS (cont.)

	Page
2.5.1 Colonization factors	27
2.5.2 Surface components	28
2.5.3 The cholera toxins	29
2.5.4 Additional virulence factors	31
2.6 Transcriptional regulation of virulence factor expression	31
2.7 Pathogenesis	34
2.8 Immunology of cholera	35
2.8.1 Nonspecific factors	36
2.8.2 Gut mucosal immunity	36
2.9 Treatment and prevention of cholera	39
2.9.1 Oral cholera vaccines	40
2.9.1.1 Whole cell-B subunit vaccine (WC-BS)	40
2.9.1.2 Live attenuated cholera vaccine	41
2.9.2 Adjuvants	43
IV MATERIALS AND METHODS	
1. Detection limit of Chole-Dot test kits performed on <i>V. cholerae</i> O1 and O139 whole cell homogenate	44
2. Sample size calculation	44
3. Culture, isolation and identification of <i>Vibrio</i> spp., <i>V. cholerae</i> O1 and <i>V. cholerae</i> O139 in clinical samples	45
4. Chole-Dot procedure for detection of <i>V. cholerae</i> O1 and <i>V. cholerae</i> O139	47
5. Polymerase chain reaction (PCR)	48
5.1 Preparation of DNA templates	48
5.2 Primer sets	49
5.3 PCR reaction mixture	49
5.4 PCR amplification	49
5.5 Detection of PCR products	49
6. Data analyses	50

CONTENTS (cont.)

	Page
6.1 Statistical analysis	50
6.2 Kappa coefficient (κ)	51
V RESULTS	
1. Detection limit of Chole-Dot test kits performed on <i>V. cholerae</i> O1 and O139 whole cell homogenate	53
2. Culture, isolation and identification of <i>Vibrio</i> spp., <i>V. cholerae</i> O1 and O139 in clinical samples	56
3. Chole-Dot for detection of <i>V. cholerae</i> O1 and <i>V. cholerae</i> O139	61
3.1 Detection of <i>V. cholerae</i> O1 antigen in the rectal swab samples using Chole-Dot MAb-based dot-ELISA	61
3.2 Detection of <i>V. cholerae</i> O139 antigen in the rectal swab samples using Chole-Dot MAb-based dot-ELISA	64
4. Detection of gene encoding some virulence factors of <i>V. cholerae</i> ; i.e. <i>ctxA</i> , <i>ctxB</i> , <i>tcpA</i> , and <i>toxR</i> by polymerase chain reaction (PCR)	68
4.1 Detection of <i>ctxA</i> , <i>ctxB</i> , and <i>tcpA</i>	68
4.2 Detection of <i>toxR</i>	73
VI DISCUSSION	76
VII CONCLUSION	80
REFERENCES	81
APPENDIX A	92
APPENDIX B	95
APPENDIX C	98
APPENDIX D	100
APPENDIX E	102
BIOGRAPHY	143