

TABLE OF CONTENTS				
Section	Sub sections		Title	Page Nos.
			List of abbreviations	
			List of figures	
			List of Tables	
			Abstract	1
Chapter -1				
Introduction				
1.1.			Microorganisms in diarrhoeal diseases	5
	1.1.1.		Diarrhoea due to <i>Vibrio</i> spp.	6
	1.1.2.		Diarrhoea due to <i>Shigella</i> spp.	8
1.2.			Antibiotics for treatment of diarrhoea caused by <i>Vibrio</i> and <i>Shigella</i> spp., and antibiotic resistance	9
	1.2.1.		Drug resistance in <i>Vibrio</i> spp.	9
	1.2.2.		Drug resistance in <i>Shigella</i> spp.	10
1.3.			Modes of action of antibiotics	11
1.4.			Mechanisms of antibiotic resistance	12
	1.4.1.		Non-genetic factors governing MDR in bacteria	12
	1.4.2.		Genetic factors/mechanisms governing MDR in bacteria	13
1.5.			Mobile genetic elements	14
	1.5.1.		Integrans	15
		1.5.1.1.	Structure of integrans	15
		1.5.1.2.	Types of integrans	17
		1.5.1.3.	Gene cassettes in the integron	21
		1.5.1.4.	Toxin- Antitoxin (TA) genes	22
	1.5.2.		Integrative and Conjugative Elements	24
		1.5.2.1.	SXT element	25
	1.5.3.		Plasmids	27
		1.5.3.1.	Features and characteristics of resistance plasmids	28
1.6.			Mobile genetic elements imparting drug resistance in <i>Vibrio</i> and <i>Shigella</i>	28
	1.6.1		Mobile genetic elements in <i>Vibrio</i> spp.	29
	1.6.2		Mobile genetic elements in <i>Shigella</i> spp.	30

1.7.			Rationale of the study	36
1.8.			Objectives of the study	36
Chapter -2				
Materials and Methods				
2.1.			Bacterial isolates	39
2.2.			Determination of antibiotic susceptibility	40
	2.2.1.		Disc diffusion method	40
		2.2.1.1.	Antibiotic susceptibility test for <i>Vibrio fluvialis</i> and <i>Shigella</i> isolates	41
	2.2.2.		Minimal inhibitory concentration assays	42
2.3.			Transferability of mobile genetic elements	42
	2.3.1.		Transformation	42
		2.3.1.1.	Preparation of electrocompetent (EC) cells	42
		2.3.1.2.	Electroporation in <i>E. coli</i>	43
	2.3.2.		Bacterial conjugation	43
		2.3.2.1.	Conjugation in <i>Vibrio fluvialis</i> isolates of 2006	44
		2.3.2.2.	Conjugation in <i>Vibrio fluvialis</i> BD146 of 2002	44
		2.3.2.3.	Conjugation in <i>Shigella</i> isolates	45
2.4.			Pulsed-field gel electrophoresis (PFGE)	45
	2.4.1.		Agarose plug preparation for PFGE	45
	2.4.2.		Restriction digestion of DNA in agarose plugs	46
	2.4.3.		Agarose gel electrophoresis for PFGE	47
	2.4.4.		PFGE gel analysis	48
2.5.			DNA isolation from the clinical isolates	48
	2.5.1.		Genomic DNA isolation	48
	2.5.2.		Plasmid DNA isolation from the clinical isolates/transformants/transconjugants	49
		2.5.2.1.	Plasmid preparation by alkaline-lysis method	49
		2.5.2.2.	Plasmid DNA isolation using Qiagen plasmid purification kit	49
2.6.			Agarose gel analysis	49
2.7.			PCR screening of mobile genetic elements	50
2.8.			Amplification of topoisomerases and mutation analysis	52
2.9.			Preparation of DNA fragments for sequencing	53

	2.9.1.		Gel extraction of DNA using Qiagen gel extraction kit	53
	2.9.2.		Purification of PCR product using Qiagen Qiaquick PCR purification kit	54
	2.9.3.		TA cloning of variable region of integrons	54
	2.9.4.		Analysis of TA clone	55
2.10.			DNA sequencing, sequence analysis and GenBank submissions	55
2.11.			Efflux pump assay	58
2.12.			Expression of the putative integrase gene in <i>Vibrio fluvialis</i> BD146	58
	2.12.1.		Total RNA isolation from the bacteria using Qiagen RNeasy mini kit	58
	2.12.2.		RNA gel electrophoresis	59
	2.12.3.		Reverse transcriptase PCR	59
Chapter-3				
Results and Discussion				
Role of mobile genetic elements in multidrug resistant <i>Vibrio fluvialis</i> isolates				
3.1.			Introduction	62
3.2.			Mechanisms of antibiotic resistance in highly drug resistant <i>Vibrio fluvialis</i> isolate BD146 of 2002	63
	3.2.1.		Results	63
		3.2.1.1.	Characterisation of BD146	63
		3.2.1.2.	Horizontal gene transfer of plasmids from <i>Vibrio fluvialis</i> BD146	63
		3.2.1.3.	Presence of integrons and SXT elements in BD146	68
		3.2.1.4.	Sequence analysis of Class 1 integron	68
		3.2.1.5.	Quinolone resistance in <i>V. fluvialis</i> BD146	71
		3.2.1.6.	Extended spectrum beta lactamases (ESBL) in <i>V. fluvialis</i> BD146	72
		3.2.1.7.	Role of efflux pump in imparting drug resistance in <i>V. fluvialis</i> BD146	73
		3.2.1.8.	Analysis of 7.5 kb plasmid BD146	74
	3.2.2.		Discussion	81
3.3.			Characterization of mobile genetic elements imparting drug resistance in <i>Vibrio fluvialis</i> isolates (2006)	85

	3.3.1.		Results	85
		3.3.1.1.	Antibiotic resistance profile of <i>Vibrio fluvialis</i> clinical isolates	85
		3.3.1.2.	Relatedness between the twelve isolates	85
		3.3.1.3.	Analysis of integrons and SXT integrase	86
		3.3.1.4.	Transfer of resistance traits by conjugation and transformation	86
		3.3.1.5.	Quinolone resistance in <i>Vibrio fluvialis</i> isolates	89
		3.3.1.6.	Role of efflux pump in MDR of <i>Vibrio fluvialis</i> isolates	90
		3.3.1.7.	Role of extended spectrum beta lactamases in <i>Vibrio fluvialis</i> isolates	91
	3.3.2.		Discussion	92
Chapter – 4 Results and Discussion Role of various mobile genetic elements in multidrug resistant <i>Shigella</i> isolates				
4.1.			Introduction	95
4.2.			Results	97
	4.2.1.		Bacterial isolates and their resistance profiles	97
	4.2.2.		Clonality in <i>Shigella</i> isolates	100
	4.2.3.		Presence of class 1 and class 2 integrons	104
	4.2.4.		Sequence analysis of integrons	108
	4.2.5.		Quinolone resistance	110
	4.2.6.		Efflux pump activity	113
	4.2.7.		Transfer of resistance by conjugation	114
4.3.			Discussion	117
			Summary and Conclusion	120
			References	125
			Appendices	157
			List of publications	
			Reprints of publications	