Section Title Chapter -1 INTRODUCTION 1.1. Gastroenteritis 1.1.1. Enteric pathogens-Vibrio spp. and Shigella spp. 1.1.1. Enteric pathogens-Vibrio spp. and Shigella spp. 1.1.1. Vibrio spp. and their outbreaks 1.1.1.2. Shigella spp. and their outbreaks 1.2. Treatment complications in Vibrio and Shigella infection due to the problem of drug resistance 1.3. Quinolone class of antibiotics 1.3.1 Targets of quinolones in bacterial cell and their mode of activity 1.3.2 Quinolone resistance mechanisms 1.3.2.1 Mutations in topoisomerase genes (chromosome-borne) 1.3.2.3. qnr genes (chromosome- and plasmid-borne) 1.3.2.4 Cir. Uinolone in the state of the problem of the plasmid-borne)	Page Nos. 2 3 3 6 7 10		
INTRODUCTION1.1.Gastroenteritis1.1.1.Enteric pathogens-Vibrio spp. and Shigella spp.1.1.1.Vibrio spp. and their outbreaks1.1.1.2.Shigella spp. and their outbreaks1.2.Treatment complications in Vibrio and Shigella infection due to the problem of drug resistance1.3.Quinolone class of antibiotics1.3.1Targets of quinolones in bacterial cell and their mode of activity1.3.2Quinolone resistance mechanisms1.3.2.1Mutations in topoisomerase genes (chromosome-borne)1.3.2.3.qnr genes (chromosome- and plasmid-borne)	3 3 6 7		
1.1.Gastroenteritis1.1.Enteric pathogens-Vibrio spp. and Shigella spp.1.1.1.Enteric pathogens-Vibrio spp. and Shigella spp.1.1.1.1.Vibrio spp. and their outbreaks1.1.1.2.Shigella spp. and their outbreaks1.2.Treatment complications in Vibrio and Shigella infection due to the problem of drug resistance1.3.Quinolone class of antibiotics1.3.1Targets of quinolones in bacterial cell and their mode of activity1.3.2Quinolone resistance mechanisms1.3.2.1Mutations in topoisomerase genes (chromosome-borne)1.3.2.2.Efflux pumps (chromosome- and plasmid-borne)1.3.2.3.qnr genes (chromosome- and plasmid-borne)	3 3 6 7		
1.1.1.Enteric pathogens-Vibrio spp. and Shigella spp.1.1.1.1.Vibrio spp. and their outbreaks1.1.1.2.Shigella spp. and their outbreaks1.2.Treatment complications in Vibrio and Shigella infection due to the problem of drug resistance1.3.Quinolone class of antibiotics1.3.1Targets of quinolones in bacterial cell and their mode of activity1.3.2Quinolone resistance mechanisms1.3.3.1Efflux pumps (chromosome- and plasmid-borne)1.3.2.3.qnr genes (chromosome- and plasmid-borne)	3 3 6 7		
1.1.1.1Vibrio spp. and their outbreaks1.1.1.1Vibrio spp. and their outbreaks1.1.1.2Shigella spp. and their outbreaks1.2.Treatment complications in Vibrio and Shigella infection due to the problem of drug resistance1.3.Quinolone class of antibiotics1.3.1Targets of quinolones in bacterial cell and their mode of activity1.3.2Quinolone resistance mechanisms1.3.2.1Mutations in topoisomerase genes (chromosome-borne)1.3.2.2.Efflux pumps (chromosome- and plasmid-borne)1.3.2.3.qnr genes (chromosome- and plasmid-borne)	3 6 7		
1.1.1.1.2.Shigella spp. and their outbreaks1.1.1.2.Treatment complications in Vibrio and Shigella infection due to the problem of drug resistance1.3.Quinolone class of antibiotics1.3.1Targets of quinolones in bacterial cell and their mode of activity1.3.2Quinolone resistance mechanisms1.3.2.1Mutations in topoisomerase genes (chromosome-borne)1.3.2.2.Efflux pumps (chromosome- and plasmid-borne)1.3.2.3.qnr genes (chromosome- and plasmid-borne)	6		
1.2.Treatment complications in <i>Vibrio</i> and <i>Shigella</i> infection due to the problem of drug resistance1.3.Quinolone class of antibiotics1.3.1Targets of quinolones in bacterial cell and their mode of activity1.3.2Quinolone resistance mechanisms1.3.2.1Mutations in topoisomerase genes (chromosome-borne)1.3.2.2.Efflux pumps (chromosome- and plasmid-borne)1.3.2.3.qnr genes (chromosome- and plasmid-borne)	7		
1.2.due to the problem of drug resistance1.3.Quinolone class of antibiotics1.3.1Targets of quinolones in bacterial cell and their mode of activity1.3.2Quinolone resistance mechanisms1.3.2.1Mutations in topoisomerase genes (chromosome-borne)1.3.2.2.Efflux pumps (chromosome- and plasmid-borne)1.3.2.3.qnr genes (chromosome- and plasmid-borne)			
1.3.1Targets of quinolones in bacterial cell and their mode of activity1.3.2Quinolone resistance mechanisms1.3.2.1Mutations in topoisomerase genes (chromosome-borne)1.3.2.2.Efflux pumps (chromosome- and plasmid-borne)1.3.2.3.qnr genes (chromosome- and plasmid-borne)	10		
1.3.1 activity 1.3.2 Quinolone resistance mechanisms 1.3.2.1 Mutations in topoisomerase genes (chromosome-borne) 1.3.2.2. Efflux pumps (chromosome- and plasmid-borne) 1.3.2.3. qnr genes (chromosome- and plasmid-borne)			
1.3.2.1Mutations in topoisomerase genes (chromosome-borne)1.3.2.2.Efflux pumps (chromosome- and plasmid-borne)1.3.2.3.qnr genes (chromosome- and plasmid-borne)	12		
1.3.2.2. Efflux pumps (chromosome- and plasmid-borne) 1.3.2.3. qnr genes (chromosome- and plasmid-borne)	15		
1.3.2.3.qnr genes (chromosome- and plasmid-borne)	15		
	17		
	18		
1.3.2.4.Ciprofloxacin modifying enzyme (AAC (6') -Ib-cr)	20		
1.3.3.Databases available for quinolone resistance genes	21		
1.4.Prevalence of quinolone resistance in Vibrio and Shigella spp.	21		
1.5. Need for this study	30		
1.6. Aims and Objectives	30		
Chapter -2			
MATERIALS AND METHODS			
2.1. Bacterial isolates/strains	33		

2.2.	Triplex PCR assay for rapid identification of <i>Vibrio</i> species	33
2.3.	Antibiogram tests for clinical isolates	36
2.4.	Minimum inhibitory concentration (MIC) assay	36
2.4.1.	MIC assay using Ezy MIC strip method	
2.4.2.	MIC assay using two-fold dilution method	37
2.4.2.1.	MIC assay of native <i>V. fluvialis</i> isolates and <i>E. coli</i> JM109 transformants	37
2.4.2.2.	MIC assay of QnrVC5 recombinants	38
2.4.2.3.	MIC assay of <i>V. fluvialis</i> isolates in presence of Peptide nucleic acid (PNA)	38
2.5.	Isolation and the analysis of genomic and plasmid DNA from the clinical isolates	39
2.5.1.	Genomic DNA isolation from the clinical isolates	39
2.5.2.	Plasmid DNA isolation from clinical isolates	40
2.5.3.	Plasmid DNA isolation using QIAGEN plasmid purification kit	41
2.6.	Polymerase chain reaction (PCR)	42
2.6.1.	PCR screening of quinolone resistance genes	42
2.6.2.	PCR amplification of QRDR regions of topoisomerase genes	42
2.7.	DNA sequencing, sequence analyis and GenBank submissions	43
2.8.	Cloning and expression of <i>qnrVC5</i> gene in heterologous <i>E. coli</i> host	43
2.8.1.	PCR amplification of <i>qnrVC5</i> gene	44
2.8.2.	Purification of PCR (<i>qnrVC5</i>) products using Qiagen QIA quick PCR purification kit	44
2.8.3.	Restriction digestion of DNA samples	48
2.8.4.	Phenol chloroform purification of restricted digested	49

2.8.5.Ligation of <i>qnrVC5</i> and pET28a2.8.6.Preparation of Electrocompetent (EC) cells	50 50
	50
2.8.7. Electroporation in <i>E. coli</i>	51
2.8.8. Preparation of master plate for transformants	51
2.8.9.Small scale preparation (Miniprep) of plasmid DNA from recombinant clones	52
2.8.10. Confirming the authenticity of recombinant pET28- qnrVC5 clones	52
2.8.10.1. PCR of <i>qnrVC5</i> gene	52
2.8.10.2. Restriction analysis of clones using <i>Nhe</i> I enzyme	52
2.8.10.3. DNA sequence analysis of clones	53
2.8.11. Preparation of calcium chloride (CaCl ₂) chemically competent cells	53
2.8.12. Transformation of <i>E. coli</i> in chemically competent cells	53
2.8.13. Expression of QnrVC5 protein	54
2.9. Total RNA isolation from bacteria using QIAGEN RNeasy mini kit	56
2.10. Expression of the putative resistance genes in the native isolates and their transformants	57
2.11. Mutant prevention concentration (MPC) assay	58
2.12 <i>In silico</i> analysis	59

Chapter -3

BACTERIAL IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY OF CLINICAL ISOLATES OF VIBRIO AND SHIGELLA SPECIES

3.1.	Introduction	61
3.2.	Results	63
3.2.1.	Bacterial strains and their identification	63

3.2.2.	Antimicrobial susceptibility of bacterial isolates	67
	Quinolone-resistance phenotypes in the isolates of	
3.2.3.	Vibrio and Shigella spp.	69
	viono and Snigeria spp.	
3.3.	Discussion	76
	Chapter – 4	
CENOI	TYPIC CHARACTERIZATION OF QUINOLONE RES	чета мт
	SOLATES OF <i>VIBRIO</i> AND <i>SHIGELLA</i> ISOLATES A	
I k	CORRELATION WITH THEIR PHENOTYPE	ND
	CORRELATION WITH THEIR FHENOTIFE	
4.1.	Introduction	80
4.2.	Results	02
4.2.	Results	82
4.0.1	PMQR determinants found in the quinolone-resistant	00
4.2.1.	isolates and their expression in the native isolates	82
4.2.2.	Mutations in QRDR region of topoisomerase genes and	84
	their role in quinolone resistance	
4.2.3.	GenBank submissions	89
4.3.	Discussion	94
	Chapter – 5	
СНАЕ	RACTERIZATION OF VIBRIO FLUVIALIS qnrVC5 G	ENE IN
	VE AND HETEROLOGOUS HOSTS: SYNERGY OF a	
	OTHER DETERMINANTS IN CONFERRING OUIN	-
	RESISTANCE	
5.1.	Introduction	97
5.2.	Results	99
	Presence of <i>qnrVC5</i> gene in <i>V. fluvialis</i> isolates,	
5.2.1.		99
3.2.1.	characterization of the gene and its flanking	99
	genetic environment	
5.0.0		
5.2.2.	Sequence analysis, homology and structure	101
	Sequence analysis, homology and structure prediction for <i>qnrVC5</i> gene/protein	101
	prediction for qnrVC5 gene/protein	101
5.2.3.	prediction for qnrVC5 gene/proteinContribution of qnrVC5 gene in quinolone	
5.2.3.	prediction for qnrVC5 gene/protein	101
5.2.3.	prediction for qnrVC5 gene/proteinContribution of qnrVC5 gene in quinolone	

	resistance of <i>E.coli</i> transformants	
5.2.5.	Expression of <i>qnrVC5</i> gene in the native <i>V</i> . <i>fluvialis</i> host and <i>E. coli</i> transformants	107
5.2.6.	Effect of PNA against <i>qnrVC5</i> gene in native <i>V</i> . <i>fluvialis</i> host	108
5.2.7.	Gene cloning and recombinant protein expression	109
5.2.8.	Elevation of MIC for quinolones in <i>qnrVC5</i> recombinants	110
5.3.	Discussion	111
	SUMMARY AND CONCLUSION	115
	REFERENCES	120
	APPENDICES	136
	LIST OF PUBLICATIONS	147
	REPRINTS OF PUBLICATIONS AND POSTERS	149