Chapter 4

Genotypic Characterization of Quinolone Resistant Isolates of Vibrio and Shigella Species and Correlation with their Phenotypes

4 Genotypic characterization of quinolone resistant isolates of *Vibrio* and *Shigella* isolates and correlation with their phenotypes

4.1. Introduction

The quinolone resistance in bacteria is mediated by the mechanisms such as mutations in the genes for drug targets, i.e. genes of DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*), efflux activity, inactivation of the drug by quinolone-modifying enzyme (AAC (6') Ib-Cr) and the target protecting Qnr protein [Kim and Hooper, 2014].

Spontaneous mutations at the quinolone-resistance-determining-regions (QRDRs) of topoisomerase genes serve as the main mechanism of resistance to quinolones as they confer moderate to high-level resistance [Jacoby, 2005]. In *E. coli*, the regions encompassing amino acids 51 to 106 in GyrA and amino acids 23 to 176 in ParC are referred as QRDR regions [Kim and Hooper, 2014]. Mutations at 83rd and 87th amino acid positions of GyrA and amino acid positions 80th and 84th of ParC are more prevalent in quinolone resistant bacteria. Mutations in GyrB and ParE occur with lesser frequency [Kim and Hooper, 2014]. Accumulation of multiple mutations in the topoisomerase genes leads to clinical failure of quinolones [Drlica and Zhao, 1997; Drilica *et al*., 2009; Kim and Hooper, 2014]. Efflux activity is another mechanism of resistance to quinolones which is intrinsic and non-specific. Efflux pumps throw the antibiotics out of the cell and provide the cell a surviving ability at suboptimal concentrations of antibiotics [Van Bambeke, 2005]. The genes for efflux pumps may either be chromosome-borne (such as NorA, NorM, AcrB, VcrM, VCH, VFH) [Morita, 2000; Van Bambeke, 2005; Piddock, 2006; Mohanty et al. 2012] or plasmid-borne (such as QepA and OqxAB) [Hansen et al. 2004; Yamane et al. 2007]. Qnr proteins belong to pentapeptide repeat family and protect DNA gyrase from quinolone action. Various allelic forms of *qnr* genes have been reported till date such as *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS* and *qnrVC* [Strahilevitz et al. 2009; Rodriguez-Martinez, 2011]. The other mechanism of quinolone resistance by a variant of

aminoglycoside acetyltransferase, AAC (6')-Ib-cr is an interesting example of promiscuous drug resistance evolved in bacteria. Aminoglycoside acetyltransferase having two amino acid changes (Trp102Arg and Asp179Tyr) confers an advantage to additionally inactivate quinolones apart from aminoglycosides [Robicsek et al. 2006].

As the factors such as Qnr, AAC (6') Ib-cr and efflux pump genes are mainly plasmid-mediated, they are referred to as plasmid-mediated quinolone resistance (PMQR) factors (Table 4.1).

Table 4.1. Description of PMQR genes screened in this study

Gene	**Description**
qnrA, qnrB, qnrC, qnrD, qnrS and qnrVC	The plasmid-borne genes encoding the pentapeptide repeat proteins which render resistance against quinolones
aac(6')Ib-cr	The plasmid-borne gene encoding a variant of aminoglycoside acetyl transferase which gives cross-resistance to fluroquinolones
qepA	The plasmid-borne gene encoding an MFS transporter that effluxes out quinolones such as norfloxacin and ciprofloxacin
oqxA	The plasmid-borne gene encoding the A subunit of OqxAB, an RND transporter, that confers resistance to oloquindox, nalidixic acid and ciprofloxacin
oqxB	The plasmid-borne gene encoding the B subunit of OqxAB, an RND transporter, that confers resistance to oloquindox, nalidixic acid and ciprofloxacin

Efflux pumps and PMQR factors generally confer low to moderate level of resistance to quinolones but in combination with mutations in the topoisomerases, they help the bacteria to achieve the clinical breakpoints of quinolone resistance

[Strahilevitz et al. 2009; Rodriguez-Martinez, 2011]. Though these factors have been well studied, there is only limited information on the role they play in *Vibrio* and *Shigella* species. The present study aimed at a thorough genotypic characterization of quinolone resistant *Vibrio* and *Shigella* isolates and the correlation of their genotypes with respect to quinolone resistance phenotypes in these major diarrhea-causing pathogens.

4.2. Results

Following phenotypic characterization of quinolone resistant clinical isolates of *Vibrio* and *Shigella* spp., they were subjected to a detailed genotypic characterization which involved deciphering of various genetic factors responsible for that quinolone resistance phenotype. All the selected quinolone resistant isolates were subjected to PCR screening of PMQR determinants and few representative quinolone resistant isolates were subjected to mutation analysis of QRDR regions of DNA gyrase and topoisomerase IV.

4.2.1. PMQR determinants found in the quinolone resistant isolates and their expression in the native isolates

All the quinolone resistant *Vibrio* and *Shigella* isolates were screened for the presence of PMQR determinants such as *qnr* genes (*qnrA, qnrB, qnrC, qnrD, qnrS* and *qnrVC*), *aac (6') Ib-cr* gene and efflux genes (*oqxAB* and *qepA*) through PCR as described in section 2.6.1. *V. fluvialis* BD146 was found to have two PMQR determinants, *qnrVC5* and *aac (6') Ib-cr* genes whereas *V. fluvialis* L10734 and L9978 possessed *qnrVC5* gene alone. The *qnrVC5* allele from the clinical isolates of *V. fluvialis* was reported for the first time from this laboratory during this study [Rajpara et al. 2009; Singh et al. 2012; Vinothkumar et al. 2016]. In the case of *V. cholerae* and *V. parahaemolyticus* isolates, none of the screened PMQR determinants were present. *S. flexneri* M11560 was found to harbor *qnrS1* gene in its plasmid preparations.

The expression of the above mentioned genes in the parent isolates was confirmed using reverse-transcription PCR, as the bands of the expected sizes i.e. 657 bp for *qnrVC5*, 608 bp for *aac(6') Ib-cr* and 657 bp for *qnrS* were observed in the corresponding isolates after reverse transcription (Fig. 4.1).

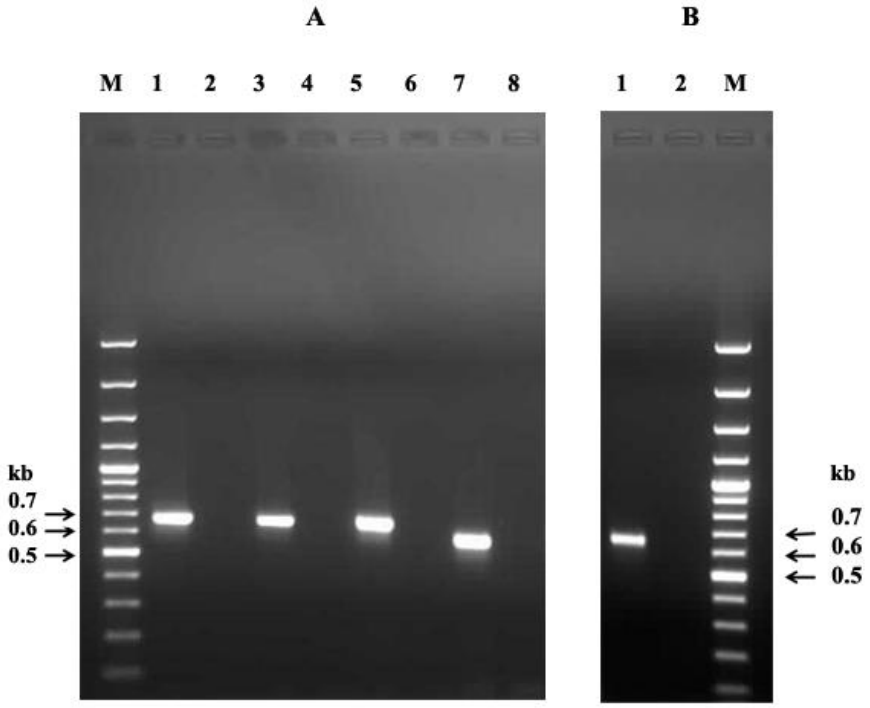

Figure 4.1. Expression of *qnrVC5, aac(6') Ib-cr* and *qnrS1* genes in the native hosts. A) Expression of *qnrVC5* and *aac (6') Ib-cr* genes in *V. fluvialis* host. Lane 1, 3, 5: RT-PCR products of *qnrVC5* transcript (657 bp) from the *V. fluvialis* hosts BD146, L10734 and L9978 respectively; Lanes 2,4, 6: Negative controls (without reverse transcription step) for RT-PCR products of *qnrVC5* transcript from the *V. fluvialis* hosts BD146, L10734 and L9978 respectively; Lane 7: RT-PCR products of *aac(6') Ib-cr* transcript (608 bp) from *V. fluvialis* BD146; Lane 8: Negative control (without reverse transcription step) for RT-PCR product of *aac(6') Ib-cr* transcript from *V. fluvialis* hosts BD146; 100 bp Gene ruler (Fermentas) was used as the marker (M). B) Expression of *qnrS1* gene in *S. flexneri* host. Lane 1: RT-PCR products of *qnrS1* transcript (657 bp) from *S. flexneri* M11560; Lane 2: Negative control (without reverse transcription step) for RT-PCR product of *qnrS1* transcript from *S. flexneri* M11560. 100 bp Gene ruler (Fermentas) was used as the marker (M).

4.2.2. Mutations in the QRDR regions of topoisomerase genes and their role in quinolone resistance

Out of nine quinolone resistant *V. cholerae* isolates (Table 4.2), four representative isolates (IDH02118, IDH02101, IDH01681 and BD81) were analyzed for the presence of mutations in the QRDR regions of topoisomerase genes as described in section 2.6.2. *V. cholerae* O1 El Tor N16961 was used as a negative control in this study (Table 4.2).

Table 4.2. Phenotype-genotype correlation in quinolone resistant isolates of *Vibrio cholerae*

S. No.	*V. cholerae*	Antibiogram profile	MIC (µg/mL)			Mutations
			NAL	NOR	CIP	
1.	IDH02365	R-NAL, I-CIP	≥256	1.5	3-4	ND
2.	IDH02233	R-NAL	≥256	-	-	ND
3.	IDH02118	R-NAL , I-NOR	≥256	0.75	-	GyrA (S83I), ParC (S85L)
4.	IDH02101	R-NAL, I-CIP	>256	-	3-4	GyrA (S83I), ParC (S85L)
5.	IDH02087	R-NAL, I-NOR	>256	2	-	ND
6.	IDH01957	R-NAL	≥256	-	-	ND
7.	IDH01738	R-NAL, I-CIP	>256	-	3-4	ND
8.	IDH01681	R-NAL	>256	0.75-1.5	3	GyrA (S83I), ParC (S85L)
9.	BD81	R-NAL	>256	-	-	GyrA (S83I), ParC (S85L)
10.	N16961*	S-NAL, NOR, CIP	0.75	0.094	0.032	None

NAL-Nalidixic acid; NOR-Norfloxacin; CIP-Ciprofloxacin; ND-Not done;
* Quinolone sensitive isolate

All the four *V. cholerae* isolates were found to have two amino acid mutations; one in GyrA (S83I) and another one in ParC (S85L). From their phenotypes, it was apparent that these mutations in *V. cholerae* isolates chiefly conferred reistance to nalidixic acid whereas contribution towards resistance to ciprofloxacin and norfloxacin was not very significant.

Table 4.3. Phenotype-genotype correlation in quinolone resistant isolates of *Vibrio fluvialis*

S.No.	***V. fluvialis***	**Antibiogram profile**	**MIC (µg/mL)**				**Mutations**	**PMQR factors**
			NAL	**NOR**	**CIP**	**OFX**		
1.	L13828*	S- NAL, NOR, CIP, OFX	1.5	0.25-0.38	0.125	0.75	None	-
2.	L13230	I-CIP	-	-	0.125	-	ND	-
3.	L98411	I-CIP	-	-	0.19	-	ND	-
4.	L10734	R-NAL I-CIP, OFX	>256	-	16	24	GyrA (S83I), ParC (S85L)	*qnrVC5*
5.	L9077	I-NAL	1.5	-	-	-	None	-
6.	L12387	R-NAL I-NOR, CIP	>256	8	8-12	-	ND	-
7.	L9978	I-CIP	-	-	0.25	-	None	*qnrVC5*
8.	L15318	R- NAL I-NOR, CIP, OFX	>256	16	16-24	16	GyrA (S83I), ParC (S85L)	-
9.	BD146	R-NAL, NOR, CIP I-OFX	>256	32	48	32	GyrA (S83I), ParC (S85L)	*qnrVC5*, *aac (6') Ib-cr*
10.	BD123	R- NAL I-NOR, CIP	>256	8-12	12	-	GyrA (S83I), ParC (S85L)	-
11.	PL78/6	R-NAL, CIP I-NOR, OFX	>256	12	24	32	GyrA (S83I), ParC (S85L)	-
12.	PL171b	R-NAL, NOR I-CIP, OFX	>256	12	16-24	32	GyrA (S83I), ParC (S85L)	-

NAL-Nalidixic acid; NOR-Norfloxacin; CIP-Ciprofloxacin; OFX-Ofloxacin; ND-Not done; * Quinolone sensitive isolate

Out of eleven selected *V. fluvialis* isolates, eight isolates (L10734, L9077, L9978, L15318, BD146, BD123, PL78/6 and PL171b) were tested for the involvement of mutations in topoisomerase genes (Table 4.3). When compared to the sensitive strain L13828, the isolates L10734, L15318, BD146, BD123, PL78/6 and PL171b possessed mutations in GyrA (S83I) and ParC (S85L) as also observed in *V. cholerae* isolates. No mutations were observed in the isolate L9978 which possessed *qnrVC5* gene and showed slightly elevated MIC of ciprofloxacin than the sensitive isolate L13828 (Table 4.3).

Table 4.4. Phenotype-genotype correlation in quinolone resistant isolates of *Vibrio parahaemolyticus*

S. No.	*V. parahaemolyticus*	Antibiogram profile	MIC (µg/mL)			Mutations
			NAL	NOR	CIP	
1.	IDH01415*	S-NAL, NOR, CIP	1	0.19	0.19	None
2.	IDH01402	I-NAL, NOR, CIP	1-1.5	0.125	0.125	ND
3.	IDH01473	I-CIP	-	-	0.25	ND
4.	IDH01998	I-CIP	-	-	0.19-0.25	ND
5.	IDH02068	I-CIP	-	-	0.19-0.25	ND
6.	IDH02189	I-NOR, CIP	-	0.125	0.25	None
7.	IDH02191	I-NAL, CIP	1.5	-	0.19	None
8.	IDH02208	I-CIP	-	-	0.19	ND

NAL-Nalidixic acid; NOR-Norfloxacin; CIP-Ciprofloxacin; ND-Not done;
* Quinolone sensitive isolate

As mentioned in chapter 3, all the seven selected *V. parahaemolyticus* isolates (IDH01402, IDH01473, IDH01998, IDH02068, IDH02189, IDH02191 and IDH02208) included in the study showed MIC similar to that of sensitive isolate IDH01415. Hence two representative test isolates, IDH02189 and IDH02191,

were checked for the presence of mutations in the topoisomerase genes along with sensitive isolate IDH01415 and none of the above tested isolates were found to have mutations in the QRDR regions of *gyrA*, *gyrB*, *parC* and *parE* genes (Table 4.4). Hence, intermediate resistance shown by these *V. parahaemolyticus* isolates in antibiogram assays and slight elevation in MIC of quinolones were presumably due to some non-specific factors such as efflux pumps.

Seven representative quinolone resistant *Shigella* isolates (four *S. flexneri*, two *S. dysenteriae* and one *S. sonnei*) and the sensitive *S. flexneri* NK 4/08, were subjected to topoisomerase mutation analysis. Among *S. flexneri* isolates, NT4966, M11560, B36, NK4/08 and NK05/08 were analysed for mutations in the QRDR regions of their topoisomerase genes. The isolate *S. flexneri* 6 (B36), which showed the highest level of resistance to all the four tested quinolones possessed three mutations, two in GyrA (S83L and D87Y) and one in ParC (E84K). The *S. flexneri* 2a isolates NT4966 and NK05/08, which showed slightly lower resistance to quinolones than the isolate B36, possessed a different combination of mutations in GyrA (S83L and D87N) and ParC (S80I). A novel mutation outside the QRDR of GyrA (H211Y) earlier reported from Bangladesh strains [Azmi et al. 2014], was also detected in *S. flexneri* 2a isolates NT4966 and NK05/08.

As both the *S. dysenteriae* isolates NK4771 and NK3898 showed different quinolone resistance phenotypes, they were subjected to topoisomerase mutation analysis. The *S. dysentaeriae* NK4771 isolate which showed high nalidixic acid resistance and intermediate level of ciprofloxacin resistance was found to possess one mutation in QRDR of GyrA (S83L). A single mutation in the 87th amino acid of QRDR of GyrA (D87Y) was found in *S. dysenteriae* NK3898 that explained the varying level of resistance to nalidixic acid exhibited by this isolate when compared to NK4771. In an earlier study, a novel mutation outside the QRDR of GyrA (V196A) was reported from the clinical isolates of *S. flexneri* and *S. dysenteriae* [Taneja et al. 2015]. Same mutation was also observed in both the *S. dysenteriae* isolates NK4771 and NK3898 tested in this study.

The *S. sonnei* isolate NK2070 which was resistant to nalidixic acid possessed a single mutation in QRDR of GyrA (D87Y).

Table 4.5. Phenotype-genotype correlation in quinolone resistant isolates of *Shigella* species

S. No.	Strain name	Antibiogram profile	MIC (µg/mL)				Mutations/ PMQR
			NAL	NOR	CIP	OFX	
1.	*S. flexneri* 1a NK 4/08*	S-NAL,NOR, CIP, OFX	1.5	0.064	0.064	0.19	None
2.	*S. flexneri* 2a NT 4966	R-NAL, NOR, CIP I-OFX	>256	16-24	24	24	GyrA (S83L, D87N, H211Y[#]), ParC (S80I)
3.	*S. flexneri* 1b M 11560	I-NAL, CIP	6	-	0.50	-	QnrS
4.	*S. flexneri* 1a NT 5120	I-NAL	1.5	-	-	-	ND
5.	*S. flexneri* 6 B 36	R- NAL, NOR, CIP, OFX	>256	32	48	≥32	GyrA (S83L, D87Y), ParC (E84K)
6.	*S. flexneri* 2a NK 05/08	R-NAL, NOR, CIP I-OFX	>256	24-32	32	32	GyrA (S83L, D87N, H211Y[#]), ParC (S80I)
7.	*S. flexneri* 3a IDH 00177	R-NAL, NOR, CIP I-OFX	>256	24	24-32	24	ND
8.	*S. flexneri* 4a NK 23/08	I-NAL	1.5	-	-	-	ND
9.	*S. dysenteriae* 12 NK 3898	R-NAL	48	-	-	-	GyrA (D87Y, V196A[#])
10.	*S. dysenteriae* 3 NK 4771	R-NAL I-CIP	>256	-	0.38	-	GyrA (S83L, V196A[#])
11.	*S. sonnei* NK 2070	R-NAL	≥256	-	-	-	GyrA (D87Y)

NAL-Nalidixic acid; NOR-Norfloxacin; CIP-Ciprofloxacin; OFX-Ofloxacin; ND-Not done; * Quinolone sensitive isolate; [#] Mutations outside the QRDR region

4.2.3. GenBank submissions

The assembled sequences of *qnrVC5*, *aac(6') Ib-cr*, *qnrS* genes and QRDR regions of *gyrA*, *gyrB*, *parC* and *parE* genes from different *Vibrio* and *Shigella* isolates were analyzed and submitted to GenBank database. These nucleotide sequences submitted to GenBank and their accession numbers are listed below (Table 4.6).

Table 4.6. GenBank submissions and their accession numbers

S. No.	Organism/ strain	Gene	Accession No.
1	*V. cholerae* IDH02118	*gyrA*	KU586854
2	*V. cholerae* IDH02118	*gyrB*	KU586870
3	*V. cholerae* IDH02118	*parC*	KU586886
4	*V. cholerae* IDH02118	*parE*	KU586902
5	*V. cholerae* IDH02101	*gyrA*	KU586853
6	*V. cholerae* IDH02101	*gyrB*	KU586869
7	*V. cholerae* IDH02101	*parC*	KU586885
8	*V. cholerae* IDH02101	*parE*	KU586901
9	*V. cholerae* IDH01681	*gyrA*	KU586852
10	*V. cholerae* IDH01681	*gyrB*	KU586868
11	*V. cholerae* IDH01681	*parC*	KU586884
12	*V. cholerae* IDH01681	*parE*	KU586900
13	*V. cholerae* BD81	*gyrA*	KU586855
14	*V. cholerae* BD81	*gyrB*	KU586871
15	*V. cholerae* BD81	*parC*	KU586887
16	*V. cholerae* BD81	*parE*	KU586903

S. No.	Organism/ strain	Gene	Accession No.
17	*V. cholerae* N16961	*gyrA*	GQ466187
18	*V. cholerae* N16961	*gyrB*	GQ466190
19	*V. cholerae* N16961	*parC*	GU326332
20	*V. cholerae* N16961	*parE*	GQ466193
21	*V. fluvialis* L13828	*gyrA*	GQ466188
22	*V. fluvialis* L13828	*gyrB*	GQ466191
23	*V. fluvialis* L13828	*parC*	GU32633
24	*V. fluvialis* L13828	*parE*	GQ466194
25	*V. fluvialis* L10734	*gyrA*	KU586904
26	*V. fluvialis* L10734	*gyrB*	KU586910
27	*V. fluvialis* L10734	*parC*	KU586916
28	*V. fluvialis* L10734	*parE*	KU586922
29	*V. fluvialis* L9077	*gyrA*	KU586905
30	*V. fluvialis* L9077	*gyrB*	KU586911
31	*V. fluvialis* L9077	*parC*	KU586917
32	*V. fluvialis* L9077	*parE*	KU586923
33	*V. fluvialis* L9978	*gyrA*	KU586906
34	*V. fluvialis* L9978	*gyrB*	KU586912
35	*V. fluvialis* L9978	*parC*	KU586918
36	*V. fluvialis* L9978	*parE*	KU586924
37	*V. fluvialis* L15318	*gyrA*	GQ466189
38	*V. fluvialis* L15318	*gyrB*	GQ466192
39	*V. fluvialis* L15318	*parC*	GU326334

S. No.	Organism/ strain	Gene	Accession No.
40	*V. fluvialis* L15318	*parE*	GQ466195
41	*V. fluvialis* BD146	*gyrA*	KU848191
42	*V. fluvialis* BD146	*gyrB*	JQ013420
43	*V. fluvialis* BD146	*parC*	JQ013421
44	*V. fluvialis* BD146	*parE*	JQ013422
45	*V. fluvialis* BD123	*gyrA*	KU586907
46	*V. fluvialis* BD123	*gyrB*	KU586913
47	*V. fluvialis* BD123	*parC*	KU586919
48	*V. fluvialis* BD123	*parE*	KU586925
49	*V. fluvialis* PL78/6	*gyrA*	KU586908
50	*V. fluvialis* PL78/6	*gyrB*	KU586914
51	*V. fluvialis* PL78/6	*parC*	KU586920
52	*V. fluvialis* PL78/6	*parE*	KU586926
53	*V. fluvialis* PL171b	*gyrA*	KU586909
54	*V. fluvialis* PL171b	*gyrB*	KU586915
55	*V. fluvialis* PL171b	*parC*	KU586921
56	*V. fluvialis* PL171b	*parE*	KU586927
57	*V. parahaemolyticus* IDH01473	*gyrA*	KU586849
58	*V. parahaemolyticus* IDH01473	*gyrB*	KU586865
59	*V. parahaemolyticus* IDH01473	*parC*	KU586881
60	*V. parahaemolyticus* IDH01473	*parE*	KU586897
61	*V. parahaemolyticus* IDH02189	*gyrA*	KU586850
62	*V. parahaemolyticus* IDH02189	*gyrB*	KU586866

S. No.	Organism/ strain	Gene	Accession No.
63	*V. parahaemolyticus* IDH02189	*parC*	KU586882
64	*V. parahaemolyticus* IDH02189	*parE*	KU586898
65	*V. parahaemolyticus* IDH02191	*gyrA*	KU586851
66	*V. parahaemolyticus* IDH02191	*gyrB*	KU586867
67	*V. parahaemolyticus* IDH02191	*parC*	KU586883
68	*V. parahaemolyticus* IDH02191	*parE*	KU586899
69	*S. flexneri* 2a NT 4966	*gyrA*	KU586840
70	*S. flexneri* 2a NT 4966	*gyrB*	KU586856
71	*S. flexneri* 2a NT 4966	*parC*	KU586872
72	*S. flexneri* 2a NT 4966	*parE*	KU586888
73	*S. flexneri* 1b M 11560	*gyrA*	KU586841
74	*S. flexneri* 1b M 11560	*gyrB*	KU586857
75	*S. flexneri* 1b M 11560	*parC*	KU586873
76	*S. flexneri* 1b M 11560	*parE*	KU586889
77	*S. flexneri* 6 B 36	*gyrA*	KU586842
78	*S. flexneri* 6 B 36	*gyrB*	KU586858
79	*S. flexneri* 6 B 36	*parC*	KU586874
80	*S. flexneri* 6 B 36	*parE*	KU586890
81	*S. flexneri* 1a NK 4/08	*gyrA*	KU586843
82	*S. flexneri* 1a NK 4/08	*gyrB*	KU586859
83	*S. flexneri* 1a NK 4/08	*parC*	KU586875
84	*S. flexneri* 1a NK 4/08	*parE*	KU586891
85	*S. flexneri* 2a NK 05/08	*gyrA*	KU586844

S. No.	**Organism/ strain**	**Gene**	**Accession No.**
86	*S. flexneri* 2a NK 05/08	*gyrB*	KU586860
87	*S. flexneri* 2a NK 05/08	*parC*	KU586876
88	*S. flexneri* 2a NK 05/08	*parE*	KU586892
89	*S. dysenteriae* 12 NK3898	*gyrA*	KU586846
90	*S. dysenteriae* 12 NK3898	*gyrB*	KU586862
91	*S. dysenteriae* 12 NK3898	*parC*	KU586878
92	*S. dysenteriae* 12 NK3898	*parE*	KU586894
93	*S. dysenteriae* 3 NK 4771	*gyrA*	KU586847
94	*S. dysenteriae* 3 NK 4771	*gyrB*	KU586863
95	*S. dysenteriae* 3 NK 4771	*parC*	KU586879
96	*S. dysenteriae* 3 NK 4771	*parE*	KU586895
97	*S. sonnei* NK 2070	*gyrA*	KU586848
98	*S. sonnei* NK 2070	*gyrB*	KU586864
99	*S. sonnei* NK 2070	*parC*	KU586880
100	*S. sonnei* NK 2070	*parE*	KU586896
101	*S. flexneri* 1b M 11560	*qnrS1*	KU848190
102	*V. fluvialis* L10734	*qnrVC5*	JN571550
103	*V. fluvialis* L9978	*qnrVC5*	JN571549
104	*V. fluvialis* BD146	*qnrVC5*	JN408080
105	*V. fluvialis* pBD146	*Plasmid*	EU574928
106	*V. fluvialis* BD146	*aac(6')-Ib-cr*	JQ013425

4.3. Discussion

The study described in this chapter involved screening of quinolone resistance determining genes in all the selected quinolone resistant isolates and detection of mutations in the QRDR regions of topoisomerase genes in few representative phenotypes. The results revealed that mutations S83I in GyrA and S85L in ParC were predominantly responsible for nalidixic acid resistance in the clinical isolates of *V. cholerae*. This finding was also supported by previous studies where the same two mutations were shown to contribute chiefly to nalidixic acid resistance [Ismail et al. 2011 and Ismail et al. 2013]. In another study, it was demonstrated that though GyrA (S83I) and ParC (S85L) mutations in *V. cholerae* isolates showed resistance to nalidixic acid, the introduction of additional mutations such as D87N in GyrA and D420N or P439S in ParE increased the spectrum of resistance to fluoroquinolones also [Zhou et al. 2013].

However, the similar mutations, GyrA (S83I) and ParC (S85L), rendered resistance to wide range of quinolones in *V. fluvialis* isolates. This observation was corroborated by a previous study on this species where the same mutations conferred reduced susceptibility to nalidixic acid, norfloxacin, ciprofloxacin and ofloxacin [Chowdhury et al. 2011].

Shigella spp. showed very interesting and varying mutation genotypes as also evidenced by their corresponding quinolone resistance phenotypes. The occurence of first mutation in GyrA which is a primary target of quinolones in Gram-negative bacteria renders cheifly nalidixic acid resistance to the organism as observed in *S. dysenteriae* NK3898 and NK4771 isolates and *S. sonnei* NK2070 isolate. This observation was in accordance with previous study on other *Shigella* spp. (*S. flexneri* and *S. boydii*) [Dutta et al. 2014]. The occurrence or accumulation of successive mutations in GyrA or ParC extended the spectrum of resistance to fluroquinolones as seen in *S. flexneri* NT4966, B36 and NK05/08 isolates. Two novel mutations were found outside the QRDR region of GyrA (H211Y and V196A) in *S. flexneri* isolates and *S. dysenteriae* isolates respectively and their role in conferring quinolone resistance needs to be established though

these mutations have also been observed by other groups [Azmi et al. 2014; Taneja et al. 2015].

PMQR genes were found either as the sole determinants for quinolone resistance as in *V. fluvialis* L9978 and *S. flexneri* M11560 or were found to act in combination with topoisomerase mutations as in *V. fluvialis* BD146 and L10734. A minimal degree of resistance to ciprofloxacin was observed in *V. fluvialis* L9978 and *S. flexneri* M11560 harbouring *qnrVC5* and *qnrS1* genes respectively. *V. fluvialis* BD146, having four quinolone resistance determinants viz. mutations S83I in GyrA and S85L in ParC, *qnrVC5* gene and *aac (6') Ib-cr* gene, was found to have an elevated MIC of quinolones when compared to *V. fluvialis* L15318 which had mutation S83I in GyrA as the only quinolone resistance determinant.

To conclude, the studies described here have clearly and vividly demonstrated that mutations in topoisomerase genes are the main players in the scenario of quinolone resistance especially nalidixic acid resistance. When assisted with other genetic factors, the resistance levels for quinolones keep getting upgraded. Similarly, PMQR determinants appeared to play a minor but additive role in deciding the fluroquinolone resistance phenotype. Additionally, the study also revealed that though PMQR genes conferred low-level resistance when present alone, they helped in the elevation of MIC of quinolones in conjunction with other genetic factors.

Among different PMQR factors described in this study, *qnrVC5* allele was first reported from this laboratory and hence it was of prime interest to decipher its role in conferring resistance to quinolones. In the next chapter, this new allele *qnrVC5* was characterized in detail for its sequence, genetic context and propensity to decrease the susceptibility for quinolones.