

Appendices

Appendix-1: Preparation of reagents and other chemicals

All the reagents were prepared in type-1 water.

1M Tris,HCl, pH 8.0	121.14 g of Tris powder (Sigma, product no. T6066) was added in 600 mL of water and pH was adjusted to 8.0 with conc. HCl. The volume was made up to 1000 mL using water and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
0.5M EDTA, pH 8.0	186.12 g of EDTA powder (Amresco, product no. 0105) was added in 600 mL water and pH was adjusted to 8.0 with NaOH. The volume was made up to 1000 mL using water and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
1X TE (Tris 10 mM; EDTA 1 mM)	10 mL of 1 M Tris, HCl (pH 8.0) and 2 mL of 0.5M EDTA (pH 8.0) were added to 800 mL of water and the volume was finally made up to 1000 mL using water.
2M MgCl ₂	19.04 g of MgCl ₂ (Sigma, product no. M8266) was dissolved in 70 mL of water and the volume was made up to 100 mL with water. The reagent was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
1M MgSO ₄	12.03 g of MgSO ₄ (Sigma, M7506) was dissolved in 70 mL of water and the volume was made up to 100 mL with water. The reagent was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
5M NaCl	29.22 g of NaCl (Amresco, product no. 0241) was dissolved in 50 mL of water and the volume was made up to 100 mL with water. The reagent was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
10N NaOH	40 g of NaOH (Amresco, product no. 0583) was dissolved in 50 mL of water and the volume was made up to 100 mL with water. The reagent was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
10% SDS	10 g of SDS (Sigma, product no. L4390) was dissolved in 50 mL of warm water and the volume was made up to 100 mL with water.
IPTG	23.8 mg of IPTG (Sigma, product no. 16758) was dissolved in 1 mL water and filter sterilized using 0.2-micron syringe filter (Fisher Scientific, product no. 190-2520).
X- Gal	80 mg of X-Gal (Sigma, product no. B9146) was dissolved in 1 mL dimethylformamide (Qualigens, product no. 12405) and filter sterilized using 0.2-micron syringe filter.
70% Ethanol	70 mL of ethanol (Amresco, product no. E193) was added to 30 mL of water and mixed well.
dNTPs (2.5 mM each)	10 µL of each dNTP (ATP, GTP, CTP, TTP) (Fermentas, product nos. R0141, R0151, R0161 and R0171) were added to make 40 µL and then the volume was made up to 400 µL using water.
1M Glucose	18.01 g of glucose (Sigma, product no. G5767) was dissolved in 70 mL of water and the volume was made up to 100 mL with water. The reagent was filter sterilized using 0.2-micron syringe filter.
50% Glycerol	50 mL of glycerol (Sigma, product no. G5516) was added to 50 mL of

	water and mixed well. The reagent was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
Primer stock solution	The lyophilized oligos were dissolved in 1X TE to get a final concentration of 1 nmol/μL stock solution. The primer working solution was made by diluting (about 20X) the stock in water.
Washing buffer (for preparation Electro-competent cells)	50 mL of glycerol was dissolved in 450 mL of water and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Appendix-2: Preparation of Media

Luria-Bertani (LB) Broth	25 g LB powder (Himedia, product no. M1245) was dissolved in 1 L water and the dissolved medium was checked for its pH 7.5 ± 0.2 . The medium was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
Luria-Bertani (LB) Agar	40 g LB agar powder (Himedia, product no. M1154) was dissolved in 1 L water and the dissolved medium was checked for its pH 7.5 ± 0.2 . The content was boiled to dissolve the medium completely. The medium was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and poured into sterile Petri plates.
Muller-Hinton Agar (MHA)	38 g MHA powder (Himedia, product no. M173) was dissolved in 1 L water and the dissolved medium was checked for its pH 7.3 ± 0.2 . The content was boiled to dissolve the medium completely. The medium was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and poured into sterile Petri plates.
Thiosulfate-Citrate-Bile salts-Sucrose (TCBS) Agar	89 g TCBS agar powder (Difco, product no. 265020) was dissolved in 1 L water and the dissolved medium was checked for its pH 8.6 ± 0.2 . The content was boiled to dissolve the medium completely. As there was no need to autoclave this medium, it was directly poured into sterile Petri plates.
MacConkey Agar	50.03 g MacConkey agar powder (Himedia, product no. MU081) was dissolved in 1 L water and the dissolved medium was checked for its pH 7.1 ± 0.2 . The content was boiled to dissolve the medium completely. The medium was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and poured into sterile Petri plates.
Xylose-Lysine Deoxycholate (XLD) agar	56.68 g XLD agar powder (Himedia, product no. M031) was dissolved in 1 L water and the dissolved medium was checked for its pH 7.4 ± 0.2 . The content was boiled to dissolve the medium completely. As there was no need to autoclave this medium, it was directly poured into sterile Petri plates.
Hektoen Enteric Agar (HEA)	72.66 g HEA powder (Himedia, product no. MU467) was dissolved in 1 L water and the dissolved medium was checked for its pH 7.5 ± 0.2 . The content was boiled to dissolve the medium completely. As there was no need to autoclave this medium, it was directly poured into sterile Petri plates.
Super Optimal Broth (SOB) media	20 g of Tryptone (Difco, product no. 211705), 5.0 g of yeast extract (Himedia, product no. RM668), 0.584 g of NaCl (Amresco, 0241) and 0.186 g KCl (Fluka, 60128) were added to 700 mL pure water and the pH was adjusted to 7.0. The volume was made up to 1000 mL and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
Super Optimal Broth with Catabolite repression (SOC) media	2 mL of 1M Glucose (sterile), 0.5 mL of 2M MgCl_2 (sterile) and 1 mL of 1M MgSO_4 (sterile) were added to 100 mL of SOB medium (sterile) at aseptic condition.

Appendix-3: Preparation of reagents for agarose gel electrophoresis

50 X TAE, pH 8.3	242 g of Tris and 18.6 g of EDTA were dissolved in 500 mL of water. 57.2 mL of glacial acetic acid (Merk, product no. AF6A560292) was added to the solution to mix well. The solution was finally made up to 1000 mL and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
1X TAE (40 mM Tris, 20 mM acetic acid and 1mM EDTA,	20 mL of 50 X TAE was diluted using water to the final volume of 1000 mL.
EtBr (10 µg/mL)	1 g Ethidium Bromide (Sigma, product no. E8751) was dissolved in 100 mL of water in a dark bottle and stored at 4°C.
6X Dye (10mM Tris-HCl (pH 7.6), 0.03% Bromophenol blue, 0.03% Xylene cyanol FF, 60% Glycerol, 60 mM EDTA)	500 µL of 1M Tris, HCl, pH 8.0, 15 mg of Bromophenol blue (Sigma, product no. B0126), 15 mg of Xylene cyanol FF (Sigma, product no.335940), 30 mL of glycerol and 6.02 mL of 0.5 M EDTA were added and then volume was made up to 50 mL with water.

Appendix-4: Genomic DNA isolation

10% CTAB	10 g of hexadecyl trimethyl ammonium bromide (CTAB) (Sigma, product no. H9151) was dissolved in 100 mL of 0.7 M NaCl.
0.7M NaCl	14 mL of 5M NaCl was dissolved in 86 mL of pure water and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
RNase A	10 mg of Ribonuclease A (from bovine pancreas) powder (Sigma, product no. R6513) was dissolved in 1 mL of sterile 0.01M Tris, HCl, pH 8.0
Proteinase K	20 mg proteinase K enzyme powder (Amresco, product no.0706) was dissolved in 1 mL of sterile water.
Chloroform: Isoamyl alcohol (24:1)	1 mL of isoamyl alcohol (Sigma, product no. 19392) was added with 24 mL of chloroform (Amresco, product no. 0757) and mixed well.
Phenol:Chloroform: Isoamyl alcohol (25:24:1)	25 mL of phenol (Sigma, product no. 77607) was added to 25 mL of chloroform:isoamyl alcohol mix (24:1) and the contents were mixed well by shaking.

Appendix-5: Preparation of reagents for plasmid DNA isolation

Solution P 1 (50mM Tris,HCl, 10 mM EDTA, 100 µg/mL)	5 mL of 1M Tris, HCl (pH 8.0), 2 mL of 0.5 M EDTA (pH 8.0) and 1.0 mL of 10 mg/mL RNaseA stock were mixed and the volume was made up to 100 mL using water.
Solution P 2 (0.2 N NaOH, 1% SDS)	2 mL of 10 N NaOH and 10 mL of 10% SDS were mixed and the volume was made up to 100 mL using water.
Solution P 3 (2.5 M potassium acetate, pH 4.8)	60 mL of 5 M potassium acetate and 11.5 mL of glacial acetic acid was mixed and the final volume was made up to 100 mL using water.
5M Potassium acetate	49.07 g of Potassium acetate (Amresco, product no. 0698) was dissolved in 70 mL of water and the volume was finally made up to 100 mL. The solution was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Appendix-6: preparation of antibiotic stock solution

Ampicillin	100 mg of ampicillin sodium salt (Himedia, product no. MB104) was dissolved in 1 mL of water. The solution was filter sterilized using 0.2-micron syringe filter and stored as aliquots at -20° C.
Kanamycin	100 mg of kanamycin acid sulphate powder (Himedia, product no. RM210) was dissolved in 1 mL of water. The solution was filter sterilized using 0.2-micron syringe filter and stored as aliquots at -20° C.
Nalidixic Acid	10 mg of nalidixic acid (Sigma, product no. N8878) was dissolved in 1 mL of 0.1N NaOH. The solution was filter sterilized using 0.2-micron syringe filter and stored as aliquots at -20° C.
Ciprofloxacin	1 mg of ciprofloxacin hydrochloride powder (Himedia, product no. RM1891) was dissolved in 1 mL of water. The solution was filter sterilized using 0.2-micron syringe filter and stored as aliquots at -20° C.
Sulphamethoxazole	100 mg sulphamethoxazole (Himedia, product no. RM5445) was dissolved in 1 mL acetone. Sterilized by filtration using 0.2-micron syringe filter and stored in aliquots at -20° C.
Trimethoprim	20 mg trimethoprim powder (Himedia, product no. CMS216) was dissolved in 1 mL benzyl alcohol and stored in aliquots at -20° C.
Streptomycin	20 mg streptomycin (Himedia, product no. CMS220) dissolved in 1 mL water. Sterilized by filtration using 0.2-micron syringe filter and stored in aliquots at -20° C.
Tetracycline	100 mg/mL tetracycline powder (Himedia, product no. CMS219) dissolved in 1 mL water. Sterilized by filtration using 0.2-micron syringe filter and stored in aliquots at -20° C.

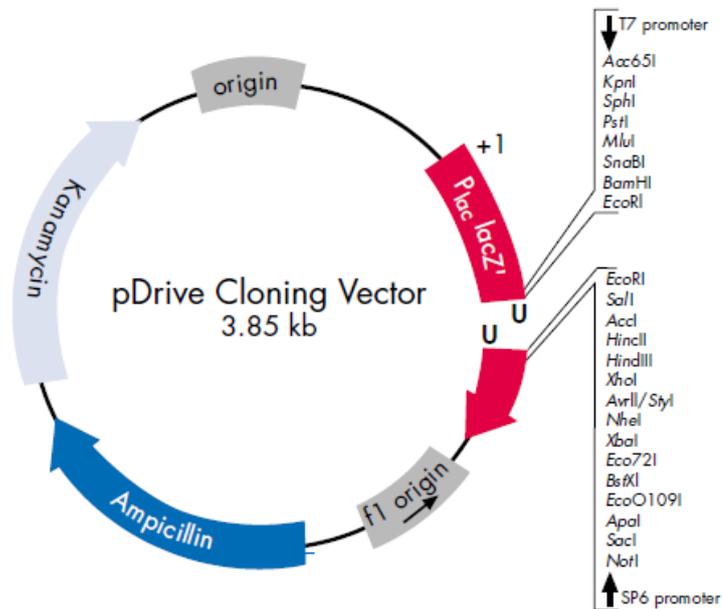
Appendix-7: preparation of reagents for Pulsed Field Gel electrophoresis

TE buffer	10 mL of 1M Tris, pH 8.0 and 2 mL of 0.5M EDTA, pH 8.0 were added to 800 mL of water and the volume was finally made up to 1000 mL using sterile water.
Cell Suspension Buffer	10 mL of 1M Tris, pH 8.0 and 20 mL of 0.5M EDTA, pH 8.0 was mixed and the volume was finally made up to 100 mL using sterile water.
Cell Lysis Buffer	25 mL of 1M Tris, pH 8.0, 50 mL of 0.5M EDTA, pH 8.0 and 50 mL 10% Sarcosyl was mixed and the volume was finally made up to 500 mL using sterile water.
10% Sarcosyl	10 g of N-Lauroylsarcosine, Sodium salt (Sigma, product no. L5125) was mixed in 50 mL sterile water and the volume was finally made up to 100 mL with sterile water.
10X TBE	108 g Tris base, 55 g Boric acid and 40 mL 0.5 M EDTA, pH 8.0 were mixed in 800 mL water and the final volume was made up to 1000 mL with water. This solution was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
0.5X TBE	50 mL of 10X TBE was diluted in 950 mL of sterile water.

Appendix-8: Preparation of reagents for RNA isolation

DEPC treated water	1 mL of Diethylpyrocarbonate (Amresco, product no. E174) was added to 1L of water and mixed vigorously. The solution was incubated at 37°C for 12 hours and autoclaved for 15 min to remove any trace of DEPC.
10X FA gel buffer (200mM MOPS, 50mM Sodium acetate and 10mM EDTA)	10.463 g of 3-[N-morpholino] propane sulfonic acid (MOPS) (Sigma, product no. M3138), 1.02 g of Sodium acetate (Sigma, product no. S2889) and 0.93 g of EDTA were dissolved in 200 mL of DEPC treated water and the pH was adjusted to 7.0 using NaOH. The final volume was made up to 250 mL using DEPC treated water.
1X FA gel buffer	100 mL of 10X FA gel buffer and 20 mL of 37% formaldehyde (Sigma, product no. F8875) were added to 880 mL of DEPC treated water and mixed well.
5X RNA loading dye	16 µL of saturated aqueous bromophenol blue solution, 80 µL of 0.5M EDTA (pH 8.0), 720 µL of 37% (12.3 M) formaldehyde, 2mL of 100% glycerol, 3.084 mL of formamide and 4 mL of 10X FA gel buffer were added and the final volume was made up to 10 mL using DEPC treated water.

Appendix-9 : Schematic representation of pDrive cloning vector



M13 reverse primer binding site → T7 promoter →

5' GAA ACA GCT ATG ACC ATG ATT ACG CCA AGC TCT AAT ACG ACT CAC TAT AGG GAA 260
 3' CTT TGT CGA TAC TGG TAC TAA TGC GGT TCG AGA TTA TGC TGA GTG ATA TCC CTT
 M T M I T P S S N T T H Y R E
 LacZ α-peptide coding sequence

Acc65I KpnI SphI PstI MluI SnaBI BamHI EcoRI

AGC TCG GTA CCA CGC ATG CTG CAG ACG CGT TAC GTA TCG GAT CCA GAA TTC GTG 314
 TCG AGC CAT GGT GCG TAC GAC GTC TGC GCA ATG CAT AGC CTA GGT CTT AAG CAC
 S S V P R M L Q T R Y V S D P E F V

PCR product

EcoRI SalI AccI HincII HindIII XhoI AvrII StyI NheI XbaI

ATU A TCT GAA TTC GTC GAC AAG CTT CTC GAG CCT AGG CTA GCT CTA GAC CAC 365
 TA UT AGA CTT AAG CAG CTG TTC GAA GAG CTC GGA TCC GAT CGA GAT CTA GAT CTG GTG
 S E F V D K L L E P R L A L D H

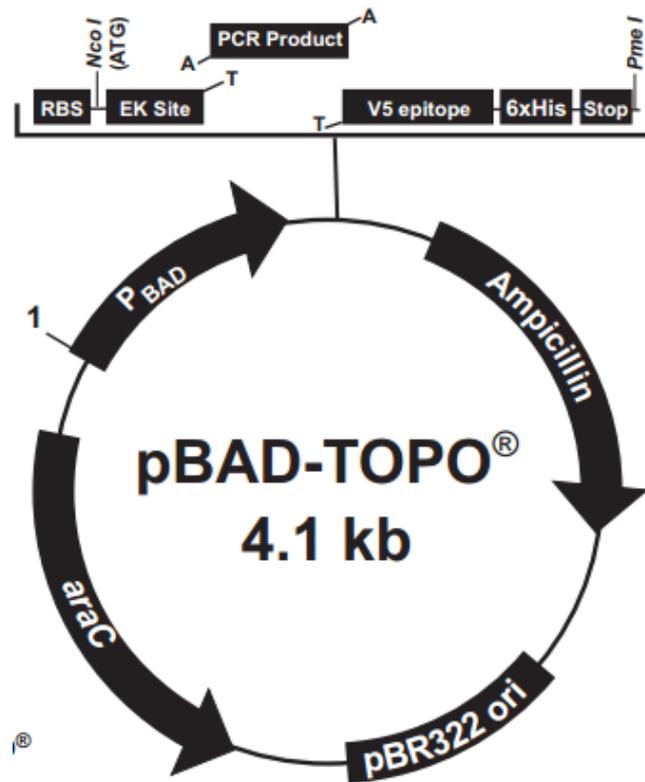
Eco72I BstXI EcoO109I ApaI SacI NotI

ACG TGT GGG GGC CCG AGC TCG CCG CCG CTG TAT TCT ATA GTG TCA CCT AAA TGG 419
 TGC ACA CCC CCG GGC TCG AGC GCC GGC GAC ATA AGA TAT CAC AGT GGA TTT ACC
 T C G G P S S R P L Y S I V S P K W

M13 forward (-20) primer binding site M13 forward (-40) primer binding site

CCG CAC AAT TCA CTG GCC GTC GTT TTA CAA CGT CGT GAC TGG GAA AAC 3' 467
 GGC GTG TTA AGT GAC CCG CAG CAA AAT GTT GCA GCA CTG ACC CTT TTG 5'
 P H N S L A V V L Q R R D W E N

Appendix-10: Schematic representation of pBAD Topo TA cloning vector



181 ATTATTTGCA CGGCGTCACA CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG
 CAP binding site
 pBAD Forward priming site
 I₁ and I₂ Region

241 ATCCTACCTG ACGCTTTTTA TCGCAACTCT C TACTGTTTC TCCATACCGG TTTTTTGGGC
 -35 -10

301 TAGAAATAAT TTTGTTTAACT TTTAAGAAGG AGATATACAT ACCC ATG GGC TCT GGA TCC
 RBS Nco I Bam II
 Met Gly Ser Gly Ser

360 GGT GAT GAC GAT GAC AAG CTC GCC CTT PCR Product AAG GGC GAG CTT GAA GGT
 Enterokinase recognition site EK cleavage site
 GAG CGG GAA TTC CCG CTC
 Gly Asp Asp Asp Asp Lys Leu Ala Leu Lys Gly Glu Leu Glu Gly

405 AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT CAT
 V5 epitope
 Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly His

456 CAT CAC CAT CAC CAT TGA GTTTAAACGG TCTCCAGCTT GGCTGTTTTG GCGGATGAGA
 Polyhistidine region Pme I
 His His His His His ***

514 GAAGATTTTC AGCCTGATAC AGATTTAAATC AGAACGCAGA AGCGGTCTGA TAAACAGAA
 pBAD Reverse priming site

574 TTTGCCTGGC GGCAGTAGCG CGGTGGTCCC ACCTGACCCC ATGCCGAAC T CAGAAGTGAA

634 ACGCCGTAGC GCCGATGGTA GTGTGGGGTC TCCCATGCG AGAGTAGGGA ACTGCCAGGC
 rrnB T₁ and T₂ transcriptional terminators

694 ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCCT TCGTTTTATC TGTTGTTTGT

Appendix-11: Antibiotic susceptibility profile and integron analysis of *Shigella flexneri* isolates

S. No	NICED No.	Strain Description	Date of Isolation	Antibiogram		Presence of class 1 integron	Presence of class 2 integron
				Resistant	Intermediate		
1	NT5120	<i>Shigella flexneri</i> (1a)	Not known	AMP, AZM, STR, TET, TRI, COT	-	+	-
2	NK 4/08	<i>Shigella flexneri</i> (1a)	08/05/08	AMP, STR, TRI, COT	KAN	+	-
3	M11560	<i>Shigella flexneri</i> (1b)	Not known	AMP, CHL, STR, TET, TRI	AZM, CIP, NAL, KAN, COT	+	+
4	NK2293	<i>Shigella flexneri</i> (1b)	2002	AMP, CHL, STR, TET, TRI	-	+	+
5	H20145	<i>Shigella flexneri</i> (1b)	2002	AMP, CHL, STR, TET, TRI, COT	NAL, KAN	+	+
6	NK2399	<i>Shigella flexneri</i> (2b)	2003	AMP, CHL, STR, TET, TRI, COT	-	+	+
7	NK2685	<i>Shigella flexneri</i> (2b)	2004	AMP, CHL, STR, TET, TRI, COT	-	+	+
8	NK 23/08	<i>Shigella flexneri</i> (4a)	02/07/08	STR	NAL, KAN, TET	+	-
9	B36	<i>Shigella flexneri</i> (6)	2007	CIP, NAL, NOR, STR, OFX, TET, TRI, COT	AZM, KAN	-	+
10	NT4966	<i>Shigella flexneri</i> (2a)	Not known	AMP, CHL, CIP, NAL, NOR, STR, TET, TRI, COT	KAN, OFX	+	+
11	NK2305	<i>Shigella flexneri</i> (2a)	2003	AMP, CHL, NAL, NOR, STR, TET, TRI, COT	CIP, OFX	+	+
12	NK2555	<i>Shigella flexneri</i> (2a)	2004	CHL, NAL, STR, TET, TRI, COT	CXM, KAN	+	+
13	NK2640	<i>Shigella flexneri</i> (2a)	2004	STR, TET, TRI, COT	CHL	+	+
14	NK2841	<i>Shigella flexneri</i> (2a)	2004	AMP, CHL, NAL, STR, TET, TRI	-	+	+
15	IDH009	<i>Shigella flexneri</i> (2a)	5/11/07	AMP, CHL, CIP, NAL, NOR, STR, TET, TRI, COT	CXM, KAN, OFX	+	+
16	IDH0067	<i>Shigella flexneri</i> (2a)	20/11/07	AMP, CHL, CIP, NAL, NOR, STR, TET, TRI, COT	KAN, OFX	+	+

17	102	<i>Shigella flexneri</i> (2a)	2007	AMP, CHL, CIP, NAL, NOR, STR, OFX, TET, TRI, COT	CXM, KAN	+	+
18	149	<i>Shigella flexneri</i> (2a)	2007	AMP, CHL, CIP, NAL, NOR, OFX, TET, TRI	AZM, CXM, KAN, STR, COT	+	+
19	NK 05/08	<i>Shigella flexneri</i> (2a)	08/05/08	AMP, CHL, CIP, NAL, NOR, STR, TET, TRI, COT	AZM, KAN, OFX	+	+
20	IDH0609	<i>Shigella flexneri</i> (2a)	3/07/08	AMP, CHL, NAL, NOR, STR, TET, TRI, COT	CIP, OFX	+	+
21	IDH0613	<i>Shigella flexneri</i> (2a)	3/07/08	AMP, CHL, CIP, NAL, NOR, STR, TET, TRI	KAN, OFX	+	+
22	IDH0654	<i>Shigella flexneri</i> (2a)	17/07/08	AMP, CHL, CIP, NAL, NOR, STR, TET, TRI, COT	AZM, CXM, OFX	+	+
23	IDH0664	<i>Shigella flexneri</i> (2a)	21/07/08	AMP, CFX, NAL, NOR, STR, TET, TRI	AZM, CHL, CIP, KAN, OFX	+	+
24	IDH0688	<i>Shigella flexneri</i> (2a)	29/07/08	AMP, CHL, NAL, STR, TET, TRI	CIP, NOR, KAN, OFX	+	+
25	195	<i>Shigella flexneri</i> (2a)	2008	AMP, CHL, CIP, NAL, STR, OFX, TET, TRI, COT	CXM, NOR, KAN	+	+
26	196	<i>Shigella flexneri</i> (2a)	2008	AMP, CHL, CIP, NAL, NOR, STR, TET, TRI, COT	AZM, KAN, OFX	+	+
27	204	<i>Shigella flexneri</i> (2a)	2008	AMP, CHL, CIP, NAL, NOR, STR, TET, TRI, COT	AZM, OFX	+	+
28	230	<i>Shigella flexneri</i> (2a)	2008	AMP, CHL, CIP, NAL, STR, TET, TRI, COT	NOR, KAN, OFX	+	+
29	393	<i>Shigella flexneri</i> (2a)	2008	AMP, CHL, CIP, NAL, NOR, STR, OFX, TET, TRI, COT	KAN	+	+
30	425	<i>Shigella flexneri</i> (2a)	2008	AMP, CHL, CIP, NAL, OFX, TET, TRI, COT	CXM, NOR, KAN, STR	+	+
31	440	<i>Shigella flexneri</i> (2a)	2008	AMP, CHL, CIP, NAL, NOR, STR, OFX, TET, TRI, COT	GEN, CXM, KAN	+	+
32	IDH 00177	<i>Shigella flexneri</i> (3a)	Not known	CHL, CIP, NAL, STR, TET, TRI, COT	KAN, OFX	+	-
33	NK2220	<i>Shigella flexneri</i> (3a)	2002	AMP, CHL, NAL, STR, TET, TRI, COT	KAN	+	+

34	IDH0022	<i>Shigella flexneri</i> (3a)	6/11/07	CHL, CIP, NAL, NOR, STR, TET, TRI, COT	KAN, OFX	+	-
35	IDH0043	<i>Shigella flexneri</i> (3a)	14/11/07	CHL, CIP, NAL, NOR, STR, TET, TRI, COT	OFX	+	-
36	129	<i>Shigella flexneri</i> (3a)	2007	NAL	-	-	-
37	IDH0787	<i>Shigella flexneri</i> (3a)	26/08/08	CHL, NAL, STR, TET, TRI, COT	-	+	-
38	177	<i>Shigella flexneri</i> (3a)	2008	CHL, CIP, NAL, NOR, TET, TRI	AZM, KAN, STR, OFX, COT	+	-
39	213	<i>Shigella flexneri</i> (3a)	2008	CIP, NAL, NOR	KAN, OFX	-	-
40	234	<i>Shigella flexneri</i> (3a)	2008	NAL	CIP, NOR, OFX	-	-
41	452	<i>Shigella flexneri</i> (3a)	2008	CHL, CIP, NAL, OFX, TET, TRI, COT	NOR, STR	+	-
42	593	<i>Shigella flexneri</i> (3a)	2008	CHL, CIP, NAL, NOR, OFX, TET, TRI, COT	KAN, STR	+	-

AMP, ampicillin; AZM, azithromycin; CFX, ceftriaxone; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; CXM, cefuroxime; NAL, nalidixic acid; NOR, norfloxacin; KAN, kanamycin; STR, streptomycin; OFX, ofloxacin; TET, tetracycline; TRI, trimethoprim; COT, co-trimoxazole

Appendix-12: Antibiotic susceptibility profile and integron analysis of *Shigella sonnei* isolates

S. No	NICED No.	Strain Description	Date of Isolation	Antibiogram		Presence of class 1 integron	Presence of class 2 integron
				Resistant	Intermediate		
1	NK1825	<i>Shigella sonnei</i>	2001	NAL, STR, TET, TRI, COT	AZM, KAN	-	+
2	NK2251	<i>Shigella sonnei</i>	2002	NAL, TRI, COT	AZM, CIP, STR, KAN	-	+
3	NK 2070	<i>Shigella sonnei</i>	19/02/02	NAL, STR, TET, TRI, COT	AZM, CIP, GEN, KAN	-	+
4	NK2437	<i>Shigella sonnei</i>	2004	NAL, TRI, COT	AZM, GEN, KAN, STR	-	+
5	NK2497	<i>Shigella sonnei</i>	2004	NAL, TRI, COT	AZM, KAN, STR	-	+
6	NK2537	<i>Shigella sonnei</i>	2004	AZM, NAL, TRI, COT	GEN, KAN	-	+
7	NK-4040	<i>Shigella sonnei</i>	2004	NAL, KAN, STR, TET, TRI, COT	AZM, CIP, GEN, NOR, OFX	-	+
8	NK-4174	<i>Shigella sonnei</i>	2004	AZM, GEN, NAL, KAN, STR, TRI, COT	-	-	+
9	NK-4224	<i>Shigella sonnei</i>	2004	NAL, TRI, COT	AZM, GEN, KAN, STR	-	+
10	NK-4290	<i>Shigella sonnei</i>	2004	NAL, TET, TRI, COT	CIP, KAN, STR	-	+
11	K18323	<i>Shigella sonnei</i>	2005	AZM, GEN, NAL, STR, TET, TRI, COT	AMP, CXM, KAN	-	+
12	NK4005	<i>Shigella sonnei</i>	2005	NAL, KAN, STR, TET, TRI, COT	AZM, CIP, GEN	-	+
13	NK4010	<i>Shigella sonnei</i>	2005	NAL, STR, TRI, COT	AZM, GEN, KAN	-	+
14	NK-4766	<i>Shigella sonnei</i>	2005	NAL, TRI, COT	KAN, STR	-	+
15	NK-4795	<i>Shigella sonnei</i>	2005	NAL, TRI, COT	AZM, KAN	-	+
16	L1137	<i>Shigella sonnei</i>	2006	NAL, STR, TRI, COT	AMP, AZM, CHL, CXM, KAN	-	+
17	NK4199	<i>Shigella sonnei</i>	2006	NAL, KAN, STR	AZM, CIP, GEN	-	-

18	NK4219	<i>Shigella sonnei</i>	2006	NAL, TRI, COT	AZM, KAN	-	+
19	NK4465	<i>Shigella sonnei</i>	2006	NAL, TRI, COT	KAN, STR	-	+
20	NK-4971	<i>Shigella sonnei</i>	2006	NAL, KAN, STR, TET, TRI, COT	CIP	-	+
21	NK4675	<i>Shigella sonnei</i>	2007	NAL, STR, TET, TRI, COT	AZM, CFX, GEN, KAN	-	+
22	NK4846	<i>Shigella sonnei</i>	2007	AZM, NAL	CFX, CIP, STR, GEN, KAN, TET	-	+
23	NK4888	<i>Shigella sonnei</i>	2007	AZM, NAL, STR, TET, TRI, COT	CFX, GEN, KAN	-	+
24	IDH0741	<i>Shigella sonnei</i>	13/08/08	GEN, NAL, NOR, STR, TET, TRI, COT	AMP, AZM, CIP, CXM, KAN, OFX	-	+
25	IDH0734	<i>Shigella sonnei</i>	12/08/08	NAL, NOR, KAN, STR, TRI, COT	AZM, CIP, OFX	+	+
26	IDH0796	<i>Shigella sonnei</i>	27/08/08	AZM, GEN, NAL, NOR, KAN, STR, TRI, COT	CFX, CIP, OFX	-	+
27	IDH988	<i>Shigella sonnei</i>	11/10/08	AZM, NAL, NOR, TRI, COT	CIP, GEN, KAN, STR, OFX	-	+
28	IDH1170	<i>Shigella sonnei</i>	13/11/08	GEN, NAL, NOR, STR, OFX, TRI, COT	AZM, CIP, KAN	-	+
29	IDH1219	<i>Shigella sonnei</i>	27/11/08	NAL, NOR, TRI, COT	CIP, KAN, STR, OFX	-	+
30	445	<i>Shigella sonnei</i>	2008	NAL, TRI, COT	AZM, KAN, STR	-	+
31	968	<i>Shigella sonnei</i>	2008	NAL, STR, TRI, COT	CIP, GEN, NOR, KAN, OFX, TET	-	+
32	1328	<i>Shigella sonnei</i>	2009	CIP, GEN, NAL, NOR, KAN, STR, OFX, TET, TRI, COT	AZM	-	+
33	1490	<i>Shigella sonnei</i>	2009	AZM, CIP, GEN, NAL, KAN, STR, OFX, TET, TRI, COT	NOR	-	+
34	2340	<i>Shigella sonnei</i>	2009	NAL, KAN, TRI, COT	AZM, CIP, GEN, NOR, STR, OFX	-	+
35	IDH1541	<i>Shigella sonnei</i>	25/03/09	NAL, NOR, STR, TET, TRI, COT	AZM, CIP, KAN, OFX	-	+
36	IDH1690	<i>Shigella sonnei</i>	22/04/09	NAL, NOR, TRI, COT	AZM, CIP, KAN, OFX	-	+
37	IDH1694	<i>Shigella sonnei</i>	23/04/09	CIP, NAL, NOR, OFX, TRI, COT	AZM, GEN, KAN, STR	-	+

38	IDH2343	<i>Shigella sonnei</i>	3/09/09	NAL, NOR, KAN, STR, TET, TRI, COT	AZM, CIP, GEN, OFX	-	+
39	IDH2347	<i>Shigella sonnei</i>	3/09/09	CIP, NAL ,NOR, KAN, STR, TET, OFX, TRI, COT	AZM, GEN	-	+
40	IDH2969	<i>Shigella sonnei</i>	26/05/10	NAL, STR, TET, TRI, COT	CIP, NOR, KAN, OFX	-	+
41	2969	<i>Shigella sonnei</i>	2010	NAL, STR, TET, TRI, COT	AZM, CIP, GEN, NOR, KAN, OFX	-	+
42	S22	<i>Shigella sonnei</i>	Not known	NAL, STR, TRI, COT	CXM, KAN	-	+

AMP, ampicillin; AZM, azithromycin; CFX, ceftriaxone; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; CXM, cefuroxime; NAL, nalidixic acid; NOR, norfloxacin; KAN, kanamycin; STR, streptomycin; OFX, ofloxacin; TET, tetracycline; TRI, trimethoprim; COT, co-trimoxazole