APPENDIX - I

LIST OF MATERIALS

A. Equipments

1. Autoclave - Life steriware, India
2. Electric balance - Explorer
3. Hot air oven - Indoexim
4. Incubator- Indoexim
5. Microscope - Olompus
6. pH meter – Hanna (H198107)
7. Refrigerator - Godrej
8. Water double distillation plant - JSGW
9. Micropipette – Status, Thermoelectron
10. Membrane filtration apparatus - Millipore

B. Glassware

1. Beakers
2. Burettes
3. Conical flask
4. Glass rods
5. Graduated cylinder
6. Microscopic slides
7. Micropippettes
8. Petri dishes
9. Pippettes
10. Reagent bottles
11. Screw capped test tubes
12. Test tubes

C. Miscellaneous

1. Aluminum foil
2. Blotting paper samples (Sample collecting bottle)
3. Cotton roll
4. Cotton swab
5. Dropper
6. Forceps
7. Immersion oil
8. Inoculating loop
9. Lens paper
10. Labelling tape
11. Transport tray

D. Chemicals and Reagents

1. Barium Chloride
2. Conc. Hydrochloric Acid
3. Crystal Violet
4. Disodium Salt of EDTA
5. Ethanol
6. Gram's Iodine
7. Hydrogen Peroxide
8. Iodine
9. Isoamyl Alcohol
10. Kovac's Reagent
11. Lysol
12. Magnesium Salt of EDTA
13. Mercuric Iodide
14. Methyl Red
15. Paraffin
16. Potassium Permanganate
17. Safranine
18. Sodium Hydroxide
19. Sulfuric Acid
20. Tetramethyl-p-Phenylenediamine Dihydroxide (Oxidase Reagent)

E. Microbiological Media

1. Eosin Methylene Blue Agar (Hi-Media)
2. Mueller Hinton Agar (Hi-Media)
3. Nutrient Agar (Hi-Media)
4. Nutrient Broth (Hi-Media)
5. Plate Count Agar (Hi-Media)
6. Salmonella-Shigella Agar (Hi-Media)

F. Biochemical Media

1. Hugh Leifson's Agar media (Hi-Media)
2. MR-VP broth (Hi-Media)
3. Simmons Citrate agar (Hi-Media)
4. Sulphide Indole Motility Medium (Hi-Media)
5. Triple Sugar Iron Agar (Hi-Media)
6. Urea Agar Base broth (Hi-Media)

APPENDIX -II

Sample site distribution

S.no	Place	Source	Date	Sample code	Coliform	Thermotolerent coliform
1	Kritipur	Tap	02/01/11	KT1	+	+
2	Kritipur	Tap	02/01/11	KT2		+
3	Kritipur	Tap	05/01/11	KT3	+	+
4	Kritipur	Tap	05/01/11	KT4	+	+
5	Kritipur	Tap	05/01/11	KT5	+	+
6	Kupandole	Tap	09/01/11	KupT1	+	+
7	Kupandole	Tap	13/01/11	KupT2	+	
8	Kupandole	Tap	13/01/11	KupT3	+	+
9	Kupandole	Tap	13/01/11	KupT4		+
10	Jhamshikel	Tap	16/01/11	JT1		+
11	Jhamshikel	Tap	16/01/11	JT2		
12	Jhamshikel	Tap	16/01/11	JT3		
13	Jhamshikel	Tap	19/01/11	JT4	+	+
14	Jhamshikel	Tap	19/01/11	JT5	+	
15	Swambhu	Tap	24/01/11	ST1		
16	Swambhu	Tap	24/01/11	ST2		
17	Swambhu	Tap	24/01/11	ST3	+	+
18	Swambhu	Tap	27/01/11	ST4		
19	Swambhu	Tap	27/01/11	ST5		
20	Balkhu	Tap	06/02/11	BalT1	+	+
21	Balkhu	Tap	06/02/11	BalT2	+	+
22	Balkhu	Tap	10/02/11	BalT3	+	+
23	Balkhu	Tap	10/02/11	BalT4		
24	Balkhu	Tap	10/02/11	BalT5		+
25	Bhaktpur	Tap	17/02/11	BhtT1	+	+
26	Bhaktpur	Tap	17/02/11	BhtT2	+	+
27	Bhaktpur	Tap	22/02/11	BhtT3	+	+
28	Bhaktpur	Tap	22/02/11	BhtT4	+	+

29	Kritipur	Well	02/01/11	KW1	+	+
30	Kritipur	Well	02/01/11	KW2	+	+
31	Kritipur	Well	06/01/11	KW3	+	+
32	Kritipur	Well	06/01/11	KW4	+	+
33	Kupandole	Well	09/01/11	KupW1	+	+
34	Kupandole	Well	11/01/11	KupW2	+	+
35	Kupandole	Well	11/01/11	KupW3		+
36	Jhamshikel	Well	16/01/11	JW1	+	+
37	Jhamshikel	Well	16/01/11	JW2		+
38	Jhamshikel	Well	18/01/11	JW3	+	+
39	Jhamshikel	Well	18/01/11	JW4		+
40	Jhamshikel	Well	18/01/11	JW5	+	+
41	Swambhu	Well	24/01/11	SW1		
42	Swambhu	Well	27/01/11	SW2	+	
43	Swambhu	Well	27/01/11	SW3	+	+
44	Swambhu	Well	27/01/11	SW5	+	+
45	Balkhu	Well	08/02/11	BalW1	+	
46	Balkhu	Well	08/02/11	BalW2	+	+
47	Balkhu	Well	13/02/11	BalW3	+	+
48	Balkhu	Well	13/02/11	BW4	+	+
49	Bhaktpur	Well	22/02/11	BhtW1	+	+
50	Bhaktpur	Well	22/02/11	BhtW2	+	+
51	Bhaktpur	Well	27/02/11	BhtW3	+	+
52	Bhaktpur	Well	27/02/11	BhtW4		+
53	Kritipur	Spout	06/01/11	KD1		
54	Kritipur	Spout	06/01/11	KD2	+	+
55	Kritipur	Spout	06/01/11	KD3		+
56	Kupandole	Spout	09/01/11	KupD1	+	+
57	Kupandole	Spout	11/01/11	KupD2	+	+
58	Kupandole	Spout	11/01/11	KupD3	+	
59	Swambhu	spout	24/01/11	SD1	+	
60	Swambhu	spout	24/01/11	SD2	+	+

61	Balkhu	Spout	14/02/11	BalD1	+	+
62	Balkhu	Spout	15/02/11	BalD2	+	+
63	Bhaktpur	Spout	22/02/11	BhtD1	+	+
64	Bhaktpur	Spout	22/02/11	BhtD2	+	+
65	Bhaktpur	Spout	27/02/11	BhtD3	+	
66	Bhaktpur	Spout	27/02/11	BhtD4	+	+

APPENDIX -III

Temperature and pH of water samples

Sample code	Temperature	pH
KT1	12.2	6.7
KT2	12.2	6.8
KT3	12.3	7.0
KT4	12.1	7.0
KT5	12.5	6.5
KupT1	12.6	6.6
KupT2	12.9	6.5
KupT3	12.5	6.9
KupT4	12.2	7.2
JT1	12.1	6.9
JT2	12.2	7.2
JT3	12.2	7.7
JT4	14.5	7.2
JT5	12.5	7.2
ST1	12.5	7.3
ST2	14.6	7.0
ST3	13.6	7.2
ST4	13.2	7.3
ST5	13.1	7.3
BalT1	13.4	6.9
BalT2	12.6	7.2
BalT3	13.9	7.5
BalT4	13.8	7.2
BalT5	14.6	7.1
BhtT1	14.6	7.5
BhtT2	14.2	7.5
BhtT3	14.6	7.2
BhtT4	16.0	7.2
KW1	10.2	7.8
KW2	10.0	8.2
KW3	11.5	8.0
KW4	11.2	7.9
KupW1	12.5	8.2
KupW2	12.5	8.1
KupW3	12.9	8.1
JW1	12.1	7.6

JW2	12.2	7.8
JW3	11.5	7.8
JW4	12.0	7.9
JW5	13.6	7.9
SW1	13.5	8.1
SW2	13.6	8.1
SW3	13.8	8.1
SW5	13.5	7.9
BalW1	13.5	8.5
BalW2	13.9	8.2
BalW3	13.1	8.2
BW4	14.1	8.3
BhtW1	15.5	8.4
BhtW2	12.5	8.2
BhtW3	12.9	8.5
BhtW4	12.5	8.0
KD1	12.1	7.1
KD2	12.6	6.6
KD3	12.5	7.9
KupD1	12.2	7.2
KupD2	13.2	6.8
KupD3	13.6	6.9
SD1	12.2	7.8
SD2	12.6	6.5
BalD1	11.2	7.9
BalD2	12.2	8.1
BhtD1	13.6	7.6
BhtD2	15.5	7.9
BhtD3	16.0	8.1
BhtD4	15.9	8.0

APPENDIX -IV

Different types of organisms isolated from drinking water

S.no	Source	Sample code	Coliform
1	Tap	KT1	*E. coli*
2	Tap	KT3	*E. coli*
3	Tap	KT4	*Klebsiella* spp
4	Tap	KT5	*Enterobacter* spp
5	Tap	KUPT1	*Citrobacter spp*
6	Tap	KUPT2	*E. coli*
7	Tap	KUPT3	*E. coli*
8	Tap	JT4	*Enterobacter* spp
9	Tap	JT5	*Klebsiella* spp
10	Tap	ST3	*Enterobacter* spp
11	Tap	BalT1	*Citrobacter spp*
12	Tap	BalT2	*E. coli*
13	Tap	BalT3	*Klebsiella* spp
14	Tap	BhtT1	*Citrobacter spp*
15	Tap	BhtT2	*Citrobacter spp*
16	Tap	BhtT3	*E. coli*
17	Tap	BhtT4	*Enterobacter* spp
18	Well	KW1	*E. coli, Citrobacter spp*
19	Well	KW2	*E. coli, Enterobacter* spp ,*Klebsiella*
20	Well	KW3	*E. coli, Citrobacter spp, Klebsiella* spp
21	Well	KW4	*E. coli , Enterobacter* spp , *Klebsiella* spp
22	Well	KUPW1	*E. coli, Citrobacter spp, Klebsiella* spp
23	Well	KUPW2	*E. coli, Enterobacter* spp , *Klebsiella* spp
24	Well	JW1	*E. coli, Enterobacter* spp ,*Citrobacter spp*
25	Well	JW3	*E. coli, Enterobacter* spp, *Klebsiella* spp
26	Well	JW5	*Enterobacter* spp ,*citrobacter spp*
27	Well	SW2	*E. coli, Citrobacter spp, Klebsiella* spp
28	Well	SW3	*E. coli, Citrobacter spp*

29	Well	SW5	*Enterobacte* spp *r, Citrobacter spp*
30	Well	BW1	*E. coli, Enterobacter* spp, *Klebsiella* spp
31	Well	BW2	*E. coli, Enterobacter* spp, *Citrobacter spp*
32	Well	BW3	*E. coli, Citrobacter* spp, *Salmonella*Typhi
33	Well	BW4	*Enterobacter ,Klebsiella* spp, *Citrobacter spp*
34	Well	BhtW1	*E. coli, Citrobacter spp,Salmonella*Typhi
35	Well	BhtW2	*E. coli, Enterobacter* spp, *Klebsiella* spp
36	Well	BhtW3	*E. coli,Citrobacter spp, Salmonella*Typhi
37	Spout	KD2	*E. coli, Citrobacter spp, Klebsiella* spp
38	Spout	KUPD1	*E. coli, Enterobacter* spp
39	Spout	KUPD2	*E. coli, Enterobacter* spp
40	Spout	KUPD3	*Citrobacter spp, Klebsiella* spp
41	spout	SD1	*Citrobacter spp*
42	spout	SD2	*E. coli, Enterobacter* spp
43	Spout	BD1	*E. coli, Citrobacter spp*
44	Spout	BD2	*E. coli, Citrobacter spp, Klebsiella* spp
45	Spout	BhtD1	*E. coli, Enterobacter* spp, *Citrobacter spp*
46	Spout	BhtD2	*E. coli, Enterobacter* spp, *Klebsiella* spp
47	Spout	BhtD3	*E. coli, Citrobacter* spp,
48	Spout	BhtD4	*Enterobacter* spp, *Klebsiella* spp

APPENDIX - V

Chart for identification of bacterial isolates

	Biochemical Tests											
Organism							SIM			TSI		
	Catalase	Oxidase	MR	VP	Urease	Citrate	H_2S	Indole	Motility	Slant	Butt	Gas
E. coli	+	-	+	-	-	-	-	+	+	Acid	Acid	+
Klebsiella	+	-	-	+	+	+	+/-	+	+	Acid	Acid	+
Citrobacter	+	-	+	-	+	+	+	-	+	Acid	Acid	+
Enterobacter	+	-	-	+	-	+	-	-	+	Acid	Acid	+
Salmonella Typhi	+	-	+	-	-	-	+	-	+	Alkaline	Acid	-

+ve : Positive

-ve : Negative

APPENDIX – VI

WHO guideline value for bacteriological quality of drinking water

	Organism value	Unit	Guideline
A.	**Piped water supplied**		
	Treated water entering distribution system		
	Thermotolerant coliform	Number/100ml	0
	Total coliform	Number/100ml	0
	Untreated water entering distribution system		
	Thermotolerant coliform	Number/100ml	0
	Total Coliform	Number/100ml	3
	Water in the distribution system		
	Thermotolerant coliform	Number/100ml	0
	Total coliform	Number/100ml	3
B.	**Unpiped water supplies**		
	Thermotolerant coliform	Number/100ml	0
	Total coliform	Number/100ml	10

APPENDIX-VII

Zone Size Interpretative Chart of Antibiotic Susceptibility Testing

Antibiotics used	Symbol	Disc content (mcg)	Diameter of zone of inhibition in mm		
			Resistant	Intermediate	Sensitive
Amoxycillin	Am	10	13	14-17	18
Amikacin	Ak	30	14	15-16	17
Cefexime	Cfx	5	15	16-18	19
Chloramphenicol	C	30	12	13-17	18
Cotrimoxazole	Co	25	10	11-15	16
Nalidixic Acid	NA	30	13	14-18	19
Ofloxacin	Of	5	15	16-20	21
Tetracycline	T	30	14	15-18	19

APPENDIX- VIII

STATISTICAL ANALYSIS

Chi square test was used for statistical analysis.

1. Source versus Total Coliform

Crosstab				
Count				
		Total Coliform		Total
		absent	present	
Source	Tap	11	17	28
	Well	5	19	24
	Spring	2	12	14
Total		18	48	66

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.729[a]	2	.155
Likelihood Ratio	3.778	2	.151
Linear-by-Linear Association	3.416	1	.065
N of Valid Cases	66		
a. 1 cells (16.7%) have expected count less than 5. The minimum expected count is 3.82.			

2. Source verses Thermotolerent Coliform

Crosstab				
Count				
		Thermotolerent Coliform		Total
		absent	present	
Source	Tap	9	19	28
	Well	3	21	24
	Spring	4	10	14
Total		16	50	66

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.896[a]	2	.235
Likelihood Ratio	3.108	2	.211
Linear-by-Linear Association	.354	1	.552
N of Valid Cases	66		
a. 1 cells (16.7%) have expected count less than 5. The minimum expected count is 3.39.			