

Impact Assessment of On-site Sanitation System on the Quality of Groundwater in Rural Areas of Patna, Bihar

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Abstract

In rural part of Bihar Pit latrines and Septic tanks are commonly used as a constituent of onsite sanitation system. These may affect ground water by contaminating through lateral leaching during monsoon. Present study was to investigate the impact of onsite sanitation system on groundwater quality in two distantly located villages Nawada and Shekhpora of Patna district Bihar. For microbial assessment across three season, MPN technique was used for coliform density and isolated colonies on selective media were characterized through biochemical test. Further antibiotic susceptibility test was performed using kirby Bauer disk diffusion method against selected antibiotics. Result show severe faecal contamination. High MPN indices during monsoon indicating heavy leaching from nearby OSS structure. Many isolates confirm faecal contamination. AST result reveals multi drug resistive strains. Result outcomes suggest that shallow aquifer in these rural settings is significantly impacted by leaching through OSS. Presence of MDR bacteria in drinking water sources leads to substantial public health risk. These findings indicate that shallow aquifers in both villages are significantly affected by lateral leaching from OSS during rainfall. This suggests an urgent need for improved OSS design, routine groundwater monitoring and targeted public health interventions to mitigate microbial and antibiotic-resistant contamination risks.

Keywords: Coliform; Onsite Sanitation; Shallow aquifer; Most Probable Number; Groundwater; Antibiotic resistance; Multi drug resistance.

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1. Introduction

In rural Bihar peoples adopting Onsite Sanitation System for sanitation and groundwater as source of water because in these areas there is absence of central water supply and sewage system. Peoples of rural area does not follow any guidelines for establishing onsite sanitation like septic tank, soak pits near ground water resources. These may be a reason for contamination of groundwater by leaching of chemical and microbial contaminants into groundwater resources which results in mortality and morbidity [1].

The current system of septic tank developed nearly a century ago, but it has some functional inadequacies. Their function depends on many factors as design construction, climate, topography, soil profile and periodic maintenance. Peoples of rural areas show their negligence towards these factors, so the problem still exists in many rural or peri urban areas [2]. For protection of public health from pathogen contamination, it is required that onsite sanitation system exist with sufficient depth of unsaturated soil and proper horizontal distance between OSS and water source should be maintained.

In developing countries like India, the impact of OSS with different soils and hydro geological condition had not carried out in compared to developed countries [3,4]. This study undertaken to assess the impact of OSS on shallow aquifers in two villages Nawada and Shekhpura of Patna district Bihar.

2. Material methods

2.1 Sample collection

For microbial study, water samples were collected from two distantly located villages Nawada and Shekhpura at Patna district. Samples were collected in a cleaned sterile 100ml plastic container, proper sealing with parafilm and transported to Dept. of Biotechnology A. N. College Patna laboratory in ice box [5].

2.2 MPN

For estimation of total coliform counts per 100ml of samples, Most Probable Number (MPN) [6] techniques were used.

2.3 Bacterial isolation

Tubes with positive culture were subculture onto selective media MacConkey Agar, Eosin Methylene Blue (EMB) Agar.

2.4 Bacterial characterization

Bacterial isolates were characterized through biochemical test include iMVIC (Indole, Methyl red, Voges Proskauer, Citrate utilisation) test and enzymatic test (Catalase, Urease, Oxidase) [5]. Further isolates were tested for their susceptibility against selected antibiotics using Kirby Bauer Disk Diffusion method. Inhibition zone was

recorded for these antibiotics. List of antibiotics shown in Table 1.

Table 1: A table of antibiotics with abbreviations and concentration

S.NO	ABRREBEATION	ANTIBIOTICS	CONCENTRATON IN MCG
1.	C	Chloramphenicol	30
2.	E	Erythromycin	15
3.	OF	Ofloxacin	5
4.	LF	levofloxacin	5
5.	A/S	Ampicillin/Sulbactam	10/10
6.	GEN	Gentamicin	10
7.	CIP	Ciprofloxacin	5
8.	TE	Tetracycline	30

3. Result

3.1 Sample collection

The bacterial density for both villages showed significant variation between three seasons. For both village MPN values were high in several sample during monsoon.

3.2 MPN

The result of estimation of total coliform counts per 100 ml of samples, Most Probable Number (MPN) techniques for village Shekhpura and Nawada are in the table 2 and 3 respectively.

Table 2: A table showing MPN values with respective samples of village Shekhpura

<i>S.No</i>	<i>Sample no</i>	<i>MPN RESULT</i>	<i>MPN value per 100 ml</i>
1	SMI01	5-2-1	70
2	SMI02	5-5-0	240
3	SMI03	5-2-3	120
4	SMI04	5-5-3	920
5	SMI05	5-5-5	1600
6	SMI06	5-3-2	140
7	SMI07	5-2-2	94
8	SMI08	5-1-0	22
9	SMI09	5-5-5	1600
10	SMI10	5-3-4	210
11	SMI11	5-5-5	1600
12	SMI12	5-3-0	79
16	SMI13	5-5-5	1600
14	SMI14	5-0-1	31
15	SMI15	5-5-0	240
16	SMI16	5-5-0	240
17	SMI17	5-5-2	540
18	SMI18	5-5-5	1600
19	SMI19	5-3-0	79
20	SMI20	5-2-1	70

Table 3: A table showing MPN values with respective samples of village Nawada

<i>S.No</i>	<i>Sample no</i>	<i>MPN RESULT</i>	<i>MPN value per 100 ml</i>
	NMI01	5-5-1	350
2	NMI02	5-5-2	540
3	NMI03	3-0-0	8
4	NMI04	4-0-0	13
5	NMI05	3-3-1	21
6	NMI06	4-2-1	26
7	NMI07	5-2-1	70
8	NMI08	5-5-1	350
9	NMI09	5-5-5	1600
10	NMI10	5-5-0	240
11	NMI11	5-4-4	350
12	NMI12	5-4-1	170
16	NMI13	5-4-0	130
14	NMI14	5-5-0	240
15	NMI15	5-1-0	33
16	NMI16	5-1-2	63
17	NMI17	3-2-0	14
18	NMI18	4-2-1	26
19	NMI19	3-2-2	20
20	NMI20	5-5-4	1600

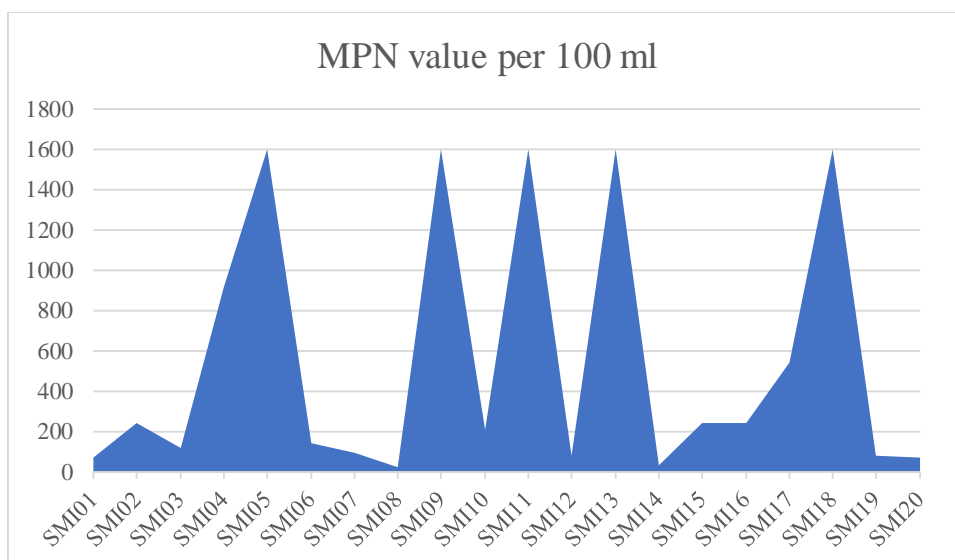


Figure 1: A graph representing MPN values with respective samples of village Shekhpura

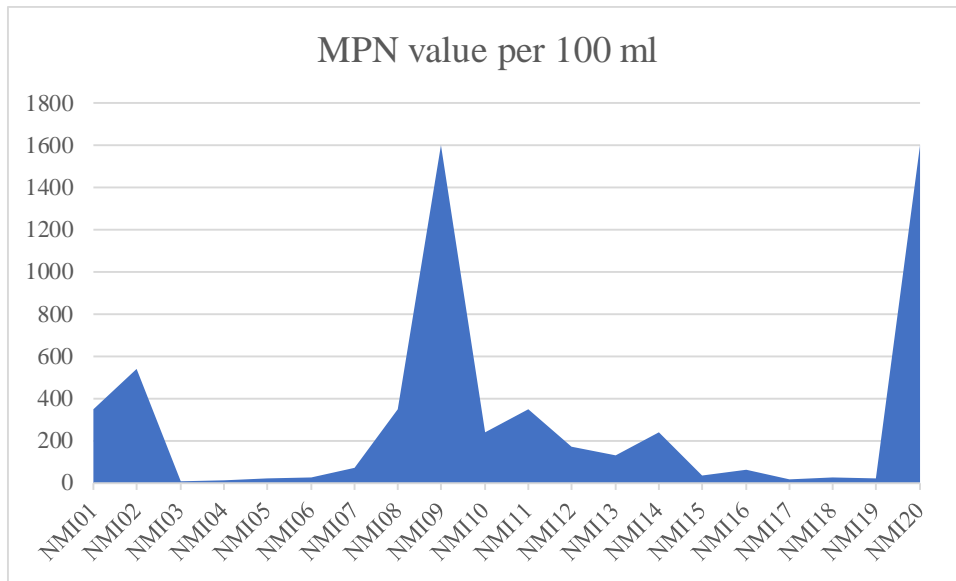


Figure 2: A graph representing MPN values with respective samples of village Nawada

3.3 Bacterial classification

The above results clearly indicate severe faecal contamination. Biochemical test confirms dominant faecal coliform like *E. coli*, *klebsiella/ Enterobacter spp.* Antibiotic susceptibility test shows high sensitivity to ciprofloxacin whereas many isolates show resistivity towards different selected antibiotics. Isolates exhibiting Multi drug resistance (MDR) pattern showed in the table 4 and 5 for village Shekhpura and Nawada respectively.

Table 4: A table representing zones of inhibition antibiotics for the samples of village Shekhpura

Isolate No.	Probable Bacterium	C	E	OF	LE	A/S	GEN	CIP	TE
SMA01	<i>Citrobacter spp.</i>	21	20	21	22	20	22	26	21
SMA02	<i>Citrobacter freundii</i>	20	13	20	21	18	22	28	22
SMA03	<i>Serratia marcescens</i>	15	20	18	20	10	25	27	6
SMA04	<i>Citrobacter spp.</i>	25	13	28	25	17	20	27	20
SMA05	<i>Escherichia coli</i>	14	13	20	23	17	17	18	10
SMA06	<i>Serratia marcescens</i>	18	10	25	33	16	30	35	12
SMA07	<i>Enterobacter aerogenes</i>	30	18	15	18	18	22	25	21
SMA08	<i>Citrobacter spp.</i>	20	10	25	22	10	20	32	13
SMA09	<i>Citrobacter freundii</i>	23	20	15	20	17	25	23	15
SMA10	<i>Escherichia coli</i>	18	12	23	20	7	23	32	15
SMA11	<i>Enterobacter spp</i>	11	6	22	24	14	22	25	9
SMA12	<i>Citrobacter spp.</i>	20	12	19	19	18	20	22	18
SMA13	<i>Aeromonas hydrophila</i>	22	0	19	18	20	17	22	20
SMA14	<i>Citrobacter freundii</i>	19	18	22	22	22	23	24	9
SMA15	<i>Aeromonas hydrophila</i>	20	19	23	18	15	20	18	12
SMA16	<i>Klebsiella oxytoca</i>	24	18	20	14	20	16	23	16
SMA17	<i>Aeromonas hydrophila</i>	22	13	19	21	18	20	25	18

SMA18	<i>Aeromonas spp.</i>	23	11	24	26	19	21	29	18
SMA19	<i>Aeromonas spp.</i>	20	13	34	33	24	26	38	22
SMA20	<i>Klebsiella oxytoca</i>	14	18	17	21	12	26	28	10
SMA21	<i>Klebsiella oxytoca</i>	13	19	23	21	0	27	29	10
SMA22	<i>Citrobacter</i>	23	17	25	25	22	23	30	17
SMA23	<i>Citrobacter freundii</i>	23	8	22	24	14	19	27	20
SMA24	<i>Citrobacter freundii</i>	18	13	32	33	20	18	40	10
SMA25	<i>Aeromonas hydrophila</i>	13	18	22	20	25	24	24	20
SMA26	<i>Enterobacter spp</i>	21	12	24	23	14	20	25	19
SMA27	<i>Aeromonas spp</i>	19	18	19	18	13	22	20	16
SMA28	<i>Aeromonas spp</i>	20	16	27	26	20	22	30	20
SMA29	<i>Aeromonas spp</i>	10	14	18	24	17	25	32	9
SMA30	<i>Aeromonas spp</i>	23	16	20	20	16	22	26	9
SMA31	<i>Aeromonas hydrophila</i>	19	9	21	22	18	26	30	8
SMA32	<i>Klebsiella oxytoca</i>	13	15	22	18	22	20	23	16
SMA33	<i>Aeromonas hydrophila</i>	24	16	21	23	22	19	24	20
SMA34	<i>Salmonella spp</i>	18	21	25	25	30	26	28	10
SMA35	<i>Aeromonas spp.</i>	22	11	28	22	24	20	36	18
SMA36	<i>Citrobacter spp</i>	15	14	21	22	11	22	26	12
SMA37	<i>Citrobacter freundii</i>	12	18	21	26	19	20	36	18
SMA38	<i>Aeromonas spp</i>	28	21	23	22	20	19	25	23
SMA39	<i>Citrobacter spp</i>	18	13	20	23	22	23	25	26
SMA40	<i>Enterobacter spp</i>	11	16	20	23	12	27	33	10
SMA41	<i>Serratia</i>	23	18	28	23	22	24	33	23
SMA42	<i>E. coli</i>	21	10	28	28	20	23	32	17

Table 5: A table representing zones of inhibition antibiotics for the samples of village Nawada

Isolate No	Probable Isolate	C	E	OF	LE	A/S	GEN	CIP	TE
NMA01	<i>Enterobacter spp.</i>	21	22	18	25	19	24	28	25
NMA02	<i>Klebsiella spp.</i>	19	23	16	26	21	17	31	19
NMA03	<i>Enterobacter spp.</i>	20	21	19	18	20	16	30	23
NMA04	<i>Escherichia coli</i>	21	19	17	16	23	20	28	18
NMA05	<i>Escherichia coli</i>	19	24	19	21	22	20	26	19
NMA06	<i>Enterobacter spp.</i>	16	26	13	19	19	16	24	17
NMA07	<i>Escherichia coli</i>	27	28	19	21	15	19	25	19
NMA08	<i>Enterobacter spp.</i>	15	24	18	23	19	12	30	18
NMA09	<i>Escherichia coli</i>	25	23	21	24	17	18	29	19
NMA10	<i>Enterobacter spp.</i>	22	27	24	21	16	19	28	21
NMA11	<i>Enterobacter spp.</i>	23	26	23	19	11	16	27	23
NMA12	<i>Escherichia coli</i>	26	24	24	20	6	24	23	14
NMA13	<i>Enterobacter spp.</i>	25	21	26	24	12	23	26	16
NMA14	<i>Escherichia coli</i>	21	23	21	26	22	21	13	12
NMA15	<i>possible Shigella spp.</i>	18	20	23	27	21	15	12	10
NMA16	<i>Escherichia coli</i>	16	19	24	21	23	10	19	21
NMA17	<i>Escherichia coli</i>	11	26	26	20	22	12	23	9
NMA18	<i>Citrobacter spp.</i>	10	27	21	23	21	13	24	13

NMA19	<i>klebsiella spp.</i>	16	23	27	15	20	24	29	23
NMA20	<i>Escherichia coli</i>	19	19	21	19	21	15	25	6
NMA21	<i>Escherichia coli</i>	10	21	23	25	26	13	26	21
NMA22	<i>Escherichia coli</i>	22	26	19	26	23	21	28	13
NMA23	<i>Citrobacter spp.</i>	25	10	20	17	25	20	27	6
NMA24	<i>Enterobacter spp.</i>	23	19	21	23	19	23	23	12
NMA25	<i>Escherichia coli</i>	24	13	18	24	18	19	24	23
NMA26	<i>Cronobacter spp.</i>	18	22	19	25	21	16	29	21
NMA27	<i>Escherichia coli</i>	19	23	16	26	12	17	28	29
NMA28	<i>Proteus spp.</i>	20	25	17	29	16	20	29	23
NMA29	<i>Enterobacter spp.</i>	24	24	23	24	18	23	23	16
NMA30	<i>Enterobacter spp.</i>	26	26	25	21	19	21	26	12
NMA31	<i>Klebsiella spp.</i>	13	27	24	26	22	19	28	10
NMA32	<i>Enterobacter spp.</i>	15	24	23	23	26	17	29	16
NMA33	<i>presumptive Cronobacter spp.</i>	18	22	16	21	29	13	25	19
NMA34	<i>presumptive Cronobacter spp.</i>	16	24	14	25	24	23	26	24
NMA35	<i>Escherichia coli</i>	6	14	16	16	23	21	19	21
NMA36	<i>Escherichia coli (atypical)</i>	6	16	23	19	20	22	23	12
NMA37	<i>Enterobacter spp.</i>	18	18	24	21	16	16	25	19
NMA38	<i>Escherichia coli</i>	12	19	26	21	18	14	29	13

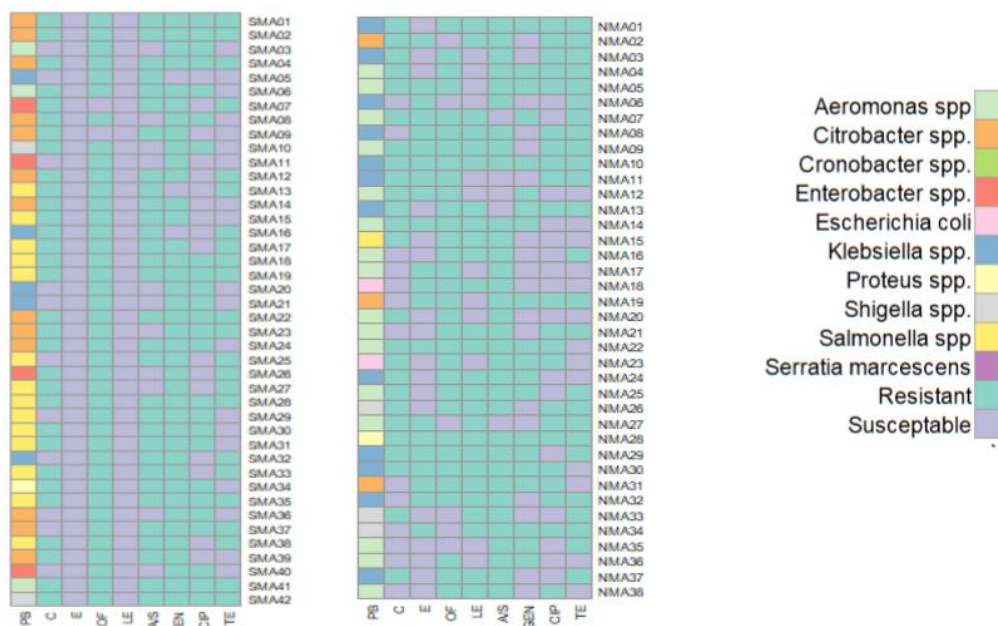


Figure 3: The heat map of antibiotic sensitivity data depicted by the ligand

4. Conclusion

Study results shows that shallow aquifers in selected villages are severely impacted by onsite sanitation system derived contamination through lateral leaching and hydraulic connectivity during rainy season. The multi drug resistance pattern among isolates indicates growing environmental reservoir of antibiotic resistance. Immediate modification in OSS design, setback distance regulation and regular microbial surveillance are important efforts to safeguard rural drinking water supplies [1,2,7].

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