Scholars Bulletin

Abbreviated Key Title: Sch Bull ISSN 2412-9771 (Print) |ISSN 2412-897X (Online) Scholars Middle East Publishers, Dubai, United Arab Emirates Journal homepage: https://saudijournals.com

Subject Category: Public Health

Effect of Community-Led Total Sanitation Intervention on Sanitation Facilities and the Bacteriological Quality of Natural Water Sources in Cross River State, Nigeria

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DOI: https://doi.org/10.36348/sb.2025.v11i04.003 | **Received:** 18.02.2025 | **Accepted:** 24.03.2025 | **Published:** 14.04.2025

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Abstract

Open defaecation (OD) has been strongly linked to high prevalence of cholera and other sanitation-related diseases. This study was conducted to assess the effect of community-led total sanitation (CLTS) on sanitation facilities and the bacteriological quality of natural water sources in Cross River State. The study design was a community-based randomized controlled trial. A multistage sampling technique was used in selecting respondents. Data were collected from 744 respondents at pre- and postintervention. A purposive sampling method was used in sampling natural water sources. Observational checklist and sterilized sample bottles were used in data collection. Data were analyzed using Statistical Product and Service Solution (SPSS version 24). Results were presented as frequencies, percentages and tables. The findings showed that respondents indicated that the major challenges of owning toilets (pre-intervention) were cost of constructing one 244 (32.8%), and distance of the toilet location 259 (34.8%) as major causes of OD practice. The same challenges were reported at post-intervention; as cost of construction 270 (36.3%) and distance of the toilet location 283 (38.0%). At pre-intervention, practice of OD was 253 (34.0%) among respondents who had no access to toilets but later reduced to 102 (13.7%) at post-intervention due to improved access to toilets. This infers that there was a statistically significant association between access to toilet and the reduction of OD practices due to the CLTS intervention. The test of hypothesis at both pre- ($\kappa^2 = 287.749$; df = 1; P-value = 0.001) and post-intervention ($\kappa^2 = 8.334$; df = 1; P-value = 0.001) was significant (P < 0.05). At pre-intervention, respondents with access to improved water sources were 119 (16.0%) which was improved to 569 (76.5%) at post-intervention, indicating that there was a statistically significant association between access to improved water sources and the reduction of sanitation-related diseases among respondents due to the CLTS intervention. Bacterial analysis showed that the total coliform and faecal coliform counts for all water samples exceeded the World Health Organisation (WHO) and the Nigerian Standard for Drinking Water Quality (NSDWQ) limits. Based on the findings, the CLTS intervention was effective in increasing knowledge of OD practice, changing wrong perceptions about OD and improving toilet ownership and maintenance. To scale-up the gains of this study, continued commitments to the eradication of OD practices from community members, community leaders, governments at all levels and NGOs is crucial.

Keywords: Community-Led Total Sanitation (CLTS), Open Defaecation (OD), Sanitation Facilities, Water Quality (Bacteriological), Cross River State (Nigeria).

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INTRODUCTION

The United Nations (UN) has positively declared sanitation as a fundamental right for every human being. However, over 2.6 billion people have been reported to have no access to improved sanitation while approximately 1.3 billion people are practicing

open defaecation, in nearly all low and middle-income countries and mostly in rural areas (Bakobie *et al.*, 2020). As a result, millions of people are suffering from water, sanitation and hygiene-related diseases such as diarrhoea, cholera, typhoid, trachoma, helminthic and skin diseases (Bakobie *et al.*, 2020). Open defaecation

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practice creates a huge public health hazard, as residents of communities where open defaecation is practiced are at increased risks of developing diarrhoeal and other sanitation-related infections (Islam *et al.*, 2018). Eradicating open defecation is the main aim of improving sanitation worldwide and it is an indicator used to measure progress towards the Sustainable Development Goal (SDG6) (Inah, *et al.*, 2023).

In low and middle-income countries, particularly Nigeria, open defaecation is a common unhygienic practice. Nigeria is one of the countries in the world with the greatest rate of open defaecation (OD), with an estimated population of over 46 million people engaging in the practice (Sawyerr & Adepoju, 2019).

Due to the decay in sanitation and hygiene, and the lack of basic amenities like pipe-borne water, there has been series of reports of cholera outbreak with many casualties in many parts of Cross River State. It was reported that in the year 2022, there were seven deaths in February and six deaths in the month of August in Odukpani L.G.A. due to cholera outbreak (The Sun, 2022 February 12; Oloyede, 2022 August 24). Similarly, in December 2022, over 22 people died and many hospitalized in Abi L.G.A. due to another cholera outbreak. (Nsa, 2022 December 18; Premium Times, 2022 December 18). Barely a month after the outbreak in Abi and Odukpani communities, other communities in the State have continued to record more casualties as 19 persons died and 286 hospitalized following an outbreak of cholera in Obubra L.G.A. of the State (Okoro, 2023 January 21). Consequently, this study assessed the availability of sanitation facilities (toilets, water sources and hand-washing facilities) during pre- and postintervention and bacteriological quality of natural water sources during pre- and post- CLTS intervention in Cross River State.

MATERIALS AND METHODS

Study Setting: The study was carried out in Cross River State, Nigeria.

Study Design: A community-based, randomized controlled trial (RCT) was conducted to evaluate the study's outcome.

Study Population

The target population for this study were adults, males and females (18 years and above) resident in Cross River State, Nigeria.

Sample Size Determination

The sample size for this study was determined using the Two Proportion Estimation Formula by Wang and Chow (2007):

Where:

- $Z_{\alpha/2}$ is the critical value of the normal distribution at $\alpha/2$

- Z_{β} is the critical value of the normal distribution at β
- P_1 and P_2 are the expected sample proportions of the two groups ($P_1 = 45\%$ (0.45); $P_2 = 55\%$ (0.55)

(Bakobie et al., 2020; UNICEF & WHO, 2020).

$$n = \underbrace{(1.96 + 0.84)^2 \times 0.45 (1 - 0.45) + 0.55(1 - 0.55)}_{(0.55 - 0.45)^2}$$

 $=218.79 \approx 219$ for each group

A design effect of 1.5 was assumed to overcome the design effect.

Then the sample size was: $219 \times 1.5 = 328.5$

The sample size was further increased by 10% to account for contingencies such as attrition or non-response

i.e., 328.5/1 - 0.1 = 328.5/0.9 = 365

The required sample size per group is = 365.

Total sample size required (for both CLTS and non-CLTS communities) was = $365 \times 2 = 730$.

For the ease of selecting equal respondents from the 24 communities, the final sample size was 744.

Sampling Procedure

This study utilized the multistage (simple random and systematic) sampling in the selection of study respondents.

Instruments for Data Collection

A semi-structured, observational checklist and sterilized sampling bottles were used to collect data and samples from natural water sources of selected communities.

Bacteriological Analysis

- The media used were: Endo agar for total coliform count and MF-C agar base for faecal coliform count.
- These were prepared based on manufacturer's instructions and sterilized in the autoclave at 121°C for 15 minutes.
- These were poured into sterile petri dishes (120ml each) and allowed to cool before inoculation.
- The glass wares and the stainless-steel filtration units used were also sterilized in the hot air oven at 150°C for 1 hour.
- The samples were shaken to mix and 100ml measured from it and filtered through the Membrane Filter (0.45 μ m pore size).
- These plates were incubated for 24 hours at 37^{0} C
- Emerging colonies after this period of incubation were enumerated using a colony counter.
- The counts were recorded accordingly.

Method of Data Entry and Analysis

Data were analyzed using the Statistical Product and Service Solutions (SPSS) software (Version 24.0). Both descriptive and inferential statistics (mean, standard deviation, chi-square, two-sample t-test, dependent sample t-test, etc.) were used to analyze the data. Relationships/associations between pre- and post-intervention were determined using Chi-square. Differences in means were determined using either A two-sample t-test or the dependent sample t-test to test if there was a difference in means comparing pre-intervention average scores to post-intervention average scores.

Ethical Considerations/Informed Consent

An Ethical clearance from the CRS Health Research Ethics Committee (CRS-HREC) was also obtained. Permission was sought for and obtained from the community leaders of the selected communities. Informed consent was sought for and obtained from every respondent.

RESULTS

Socio-Demographic Characteristics of Study Respondents

Slightly above half 386 (51.9%) of the 744 respondents were females while 358 (48.1%) were males. Most of the respondents 200 (26.9%) were within the age group 38-47 years, followed by 181 (24.3%) within the age group of 48-57 years, respondents aged 28-37 years were 165 (22.2%), followed by 103 (13.8%)

within the age group of 18-27 years and the least 95 (12.8%) were within the age group of 58 years and above. Majority 219 (29.4%) were married, followed by 212 (28.5%) singles, 131 (17.6%) were divorced, 104 (14.0%) widowed while the least 78 (10.5%) were cohabiting. Most of the respondents 279 (37.5%) had 4-6 members in their households, followed by those 222 (29.8%) who had 7-9 household members, 156 (21.0%) had 1-3 household members while the least 87 (11.7%) had 10 members and above. A greater proportion 162 (21.8%) were traders, followed by 149 (20.0%) farmers, 130 (17.5%) were civil servants, 94 (12.6%) were housewives, 65 (8.7%) were artisans, 54 (7.3%) were students 63 (8.5%) were unemployed while 27 (3.3%) did not disclose their occupations. A higher proportion of the respondents 692 (93.0%) subscribed to Christianity, 35 (4.7%) were traditionalists while only 17 (2.3%) were Muslims. Most of the respondents 339 (45.6%) had secondary education, followed by 212 (28.5%) who had tertiary education, 129 (17.3%) had primary education and 64 (8.6%) had no formal education. Most of the respondents 357 (48.0%) were earning within N30,000-N 70,000 while 265 (35.6%) earned less than \$30,000 and only 122 (16.4%) respondents earned above ¥70,000. A greater proportion 212 (28.5%) were living in mud houses with zinc roof, 150 (20.2%) lived in block houses with zinc roof/asbestos roofing sheets, this was followed by 164 (22.0%) who lived in uncompleted buildings while 134 (18.0%) lived in mud house with bamboo roof and the least 84 (11.3%) lived in wooden-made houses (Table 1).

Table 1: Socio-demographic characteristics of study respondents (n=744)

Variables	Abi(%)	Biase(%)	Yakurr(%)	Total(%)
Sex of respondents				
Females	125(16.8)	134(18.0)	127(17.1)	386(51.9)
Males	123(16.5)	114(15.3)	121(16.3)	358(48.1)
Age of respondents (years)				
18-27	27(3.6)	40(5.4)	36(4.8)	103(13.8)
28-37	49(6.6)	54(7.3)	62(8.3)	165(22.2)
38-47	77(10.3)	59(7.9)	64(8.6)	200(26.9)
48-57	66(8.9)	61(8.2)	54(7.3)	181(24.3)
58 and above	29(3.9)	34(4.6)	32(4.3)	95(12.8)
Marital status				
Married	73(9.8)	82(11.0)	64(8.6)	219(29.4)
Single	64(8.6)	75(10.1)	73(9.8)	212(28.5)
Divorced	42(5.6)	49(6.6)	40(5.4)	131(17.6)
Widowed	40(5.4)	29(3.9)	35(4.7)	104(14.0)
Cohabiting	29(3.9)	13(1.7)	36(4.8)	78(10.5)
Household size				
1-3 members	40(5.4)	52(7.0)	64(8.6)	156(21.0)
4-6 members	100(13.4)	86(11.6)	93(12.5)	279(37.5)
7-9 members	83(11.2)	75(10.1)	64(8.6)	222(29.8)
10 and above	25(3.4)	35(4.7)	27(3.6)	87(11.7)
Occupation				
Farming	70(9.4)	49(6.6)	30(4.0)	149(20.0)
Traders	47(6.3)	60(8.1)	55(7.4)	162(21.8)
Civil servants	35(4.7)	45(6.0)	50(6.7)	130(17.5)
Housewives	24(3.2)	34(4.6)	36(4.8)	94(12.6)
Artisan	19(2.6)	21(2.8)	25(3.4)	65(8.7)

			1	
Students	18(2.4)	17(2.3)	19(2.6)	54(7.3)
Unemployed	25(3.4)	17(2.3)	21(2.8)	63(8.5)
Others	10(1.3)	5(0.7)	12(1.6)	27(3.6)
Religion				
Christianity	228(30.6)	233(31.3)	231(31.0)	692(93.0)
Islam	8(1.2)	4(0.5)	5(0.7)	17(2.3)
Traditional religion	12(1.6)	11(1.5)	12(1.6)	35(4.7)
Educational level				
No formal edu.	21(2.8)	16(2.2)	44(5.9)	64(8.6)
Primary	45(6.0)	40(5.4)	93(12.5)	129(17.3)
Secondary	114(15.3)	132(17.7)	84(11.3)	339(45.6)
Tertiary	68(9.1)	60(8.1)	27(3.6)	212(28.5)
Monthly income				
Less than ¥30,000	87(11.7)	74(9.9)	104(14.0)	265(35.6)
N 30,000- N 70,000	110(14.8)	129(17.3)	118(15.9)	357(48.0)
Above ₩70,000	51(6.9)	45(6.0)	26(3.5)	122(16.4)
House type				
Mud house /bamboo roof	56(7.5)	33(4.4)	45(6.0)	134(18.0)
Mud house with zinc roof	76(10.2)	59(7.9)	77(10.3)	212(28.5)
Block house with zinc roof/asbestos roofing sheets	23(3.1)	57(7.7)	70(9.4)	150(20.2)
Uncompleted building	57(7.7)	65(8.7)	42(5.6)	164(22.0)
Wooden-made house	36(4.8)	34(4.6)	14(1.9)	84(11.3)

Latrine/Toilet Ownership and Use

The data in table 2.a showed that at the baseline phase, more than half 425 (57.1%) had no latrine/toilets and this was highest 185 (24.9%) among respondents in Biase L.G.A. and least 63 (8.5%) in Yakurr L.G.As while after the intervention, the post-intervention data showed that latrine/toilet ownership increased by 43.4% giving a total of 642 (86.3%) latrine/toilet ownership and was highest 224 (30.1%) in Yakurr L.G.A. and least 207 (27.8%) in Abi L.G.A.s respectively. The most owned latrine/toilet was pit latrine without slab 114 (15.3%) at baseline while at post-intervention, pit latrine without slap was still found to be the most owned latrine/toilet 194 (26.1%). The least 66 (8.9%) at baseline and 44 (5.9%) at post-intervention were pour flush and latrines draining to canal/stream/river respectively. At baseline, use of nearby bushes/gutters/streams was high 492 (66.1%) whose responses were same after intervention perhaps because the question was to access those who had ever involved in the act of using nearby bush/gutter/stream for defaecation (Table 2.a).

Most of the respondents 432 (58.1%) had their toilets 50 metres away from their houses (pre- and postintervention). More than 7 family members shared latrines among 304 (40.9%) respondents (pre- and postintervention). Majority of the respondents 310 (41.7%) professed defaecating in public latrines in the absence of self-owned latrines (pre- and post-intervention). Some respondents 110 (14.8%) maintained that the reasons for non-ownership of latrines is because of the high cost of owning one (pre- and post-intervention). Disposal of baby/infants' faeces was mostly 235 (31.6) done by burying at baseline but was later improved at postintervention by disposal of baby/infants' faeces in toilet 273 (36.7%). At baseline, hand washing after the use of toilet was poor 158 (21.2%) for those who do that always while it was improved at post-intervention where those who always practice hand washing after the use of toilet were 296 (39.8%). The summary of the results of this part of the study is in Table 2.a.

Table 2.a: Latrine/toilet ownership and use

	1 a	Table 2.a. Lattine/tonet ownership and use								
Variable	Baseline d	Baseline data				Post-intervention data				
	Abi	Biase	Yakurr	Total	Abi	Biase	Yakurr	Total		
Ownership of										
latrines/toilets										
Those who owned	71(9.5)	63(8.5)	185(24.9)	319(42.9)	207(27.8)	211(28.4)	224(30.1)	642(86.3)		
Those who don't own	177(23.8)	185(24.9)	63(8.5)	425(57.1)	41(5.5)	37(5.0)	24(3.2)	102(13.7)		
Types of latrine/toilets										
owned (n=319)										
Pour flush	16(1.2)	11(1.5)	39(5.2)	66(8.9)	50(6.7)	48(6.5)	51(6.9)	149(20.0)		
Pit latrine with slap	22(3.0)	15(2.0)	45(6.1)	82(11.0)	56(7.5)	52(7.0)	54(7.3)	162(21.8)		
Pit latrine without slap	23(3.9)	25(3.4)	66(8.9)	114(15.3)	57(7.7)	62(8.3)	75(10.1)	194(26.1)		
Latrine draining to	10(1.3)	12(1.6)	35(4.7)	57(7.7)	44(5.9)	49(6.6)	44(5.9)	137(18.4)		
canal/stream/river										

Use of nearby								
bush/gutter/stream								
Ever used	169(22.7)	160(21.5)	163(21.9)	492(66.1)	169(22.7)	160(21.5)	163(21.9)	492(66.1)
Never used	79(10.6)	88(11.8)	85(11.4)	252(33.9)	79(10.6)	88(11.8)	85(11.4)	252(33.9)
Distance of latrine from								
house								
Within 50 meters	56(7.5)	152(20.4)	104(14.0)	312(41.9)	56(7.5)	152(20.4)	104(14.0)	312(41.9)
More than 50 meters	192(25.8)	96(12.9)	144(19.4)	432(58.1)	192(12.9)	96(12.9)	144(19.4)	432(58.1)
Household member sharing								
latrine								
2-4 households	104(14.0)	101(13.6)	92(12.3)	297(40.0)	104(14.0)	101(13.6)	92(12.3)	297(40.0)
5-7 households	21(2.8)	53(7.1)	69(9.3)	143(19.2)	21(2.8)	53(7.1)	69(9.3)	143(19.2)
More than 7	123(16.5)	94(12.6)	87(11.7)	304(40.9)	123(16.5)	94(12.6)	87(11.7)	304(40.9)
*Place of defaecation in the	- ()	- (/		(1.1.)				(111)
absence of latrine								
Public latrine	103(13.8)	106(14.2)	101(13.6)	310(41.7)	103(13.8)	106(14.2)	101(13.6)	310(41.7)
Neighbour's latrine	49(6.6)	151(20.3)	126(16.9)	228(30.6)	49(6.6)	151(20.3)	126(16.9)	228(30.6)
Plastic bag	55(7.4)	65(8.7)	86(11.6)	206(27.7)	55(7.4)	65(8.7)	86(11.6)	206(27.7)
Dig hole/cat hole	115(15.5)	129(17.3)	161(21.6)	405(54.4)	115(15.5)	129(17.3)	161(21.6)	405(54.4)
Bush/backyard/field	182(24.5)	220(29.6)	209(28.1)	611(82.1)	182(24.5)	220(29.6)	209(28.1)	611(82.1)
*Reasons for not owning	102(2110)	220(25.0)	20)(20:1)	011(02.1)	102(2110)	220(23:0)	207(20:1)	011(02.1)
latrine								
Too expensive	37(5.0)	32(4.3)	41(5.5)	110(14.8)	37(5.0)	32(4.3)	41(5.5)	110(14.8)
No space	21(2.8)	20(2.7)	48(6.5)	89(12.0)	21(2.8)	20(2.7)	48(6.5)	89(12.0)
A lot of space to defaecate	42(5.6)	49(6.6)	40(5.4)	131(17.6)	42(5.6)	49(6.6)	40(5.4)	131(17.6)
Not a priority	19(2.6)	50(6.7)	23(3.1)	89(12.0)	19(2.6)	50(6.7)	23(3.1)	89(12.0)
*Disposal of baby/infants'			- (-)				- (- ')	
faeces								
Toilet	16(2.2)	56(7.5)	43(5.8)	115(15.5)	101(13.6)	85(11.4)	87(11.7)	273(36.7)
Garbage pit	101(13.6)	82(11.0)	48(6.5)	231(31.0)	63(8.5)	82(11.0)	69(9.3)	214(28.8)
Sine age par	101(12.0)	02(11.0)	10(0.0)	201(01.0)	05(0.5)	02(11.0)	0)().5)	21 ((20.0)
Bury	81(10.9)	85(11.4)	69(9.3)	235(31.6)	73(9.8)	56(7.5)	73(9.8)	202(27.2)
Bush	63(8.5)	52(7.0)	87(11.7)	202(27.2)	81(10.9)	52(7.0)	48(6.5)	181(24.3)
Stream	73(9.8)	65(8.7)	73(9.8)	211(28.4)	34(4.6)	65(8.7)	43(5.8)	142(19.1)
Ground/field	34(4.6)	47(6.3)	39(5.2)	120(16.1)	16(2.2)	47(6.3)	39(5.2)	102(13.7)
*Material used for anal	- 1(114)	. (2.2)	/ /	()	~ (=/	. (===)	- / (+ /	(/)
cleansing after defaecation								
Water	210(28.2)	201(27.0)	192(25.8)	603(81.0)	24(3.2)	12(1.6)	15(2.0)	51(6.9)
Leaves	16(2.2)	34(4.6)	71(9.5)	121(16.3)	6(0.8)	14(1.9)	11(1.5)	31(4.2)
Papers	62(8.3)	84(11.3)	73(9.8)	219(29.4)	12(1.6)	48(6.5)	13(1.7)	73(9.8)
Tissue	128(17.2)	126(16.9)	161(21.6)	415(55.8)	202(27.2)	171(23.0)	202(27.2)	575(77.3)
Stick	34(4.6)	31(4.2)	17(2.3)	82(11.0)	4(0.5)	3(0.4)	7(0.9)	14(1.9)
Washing of hand after	2.()	-1()	= / (=.5)	=(11.0)	.(0.0)	-(0)	. (0.7)	- ((2.7)
toilet use								
Always	41(5.5)	52(7.0)	65(8.7)	158(21.2)	105(14.1)	110(14.8)	81(10.9)	296(39.8)
Most times	31(4.2)	21(2.8)	43(5.8)	95(12.8)	71(9.5)	65(8.7)	65(8.7)	201(27.0)
Sometimes	105(14.1)	110(14.8)	59(7.9)	274(36.8)	41(5.5)	52(7.0)	59(7.9)	152(20.4)
Never	71(9.5)	65(8.7)	81(10.9)	217(29.2)	31(4.2)	21(2.8)	43(5.8)	93(12.5)
110101	11(2.2)	03(0.1)	01(10.7)	211(2).2)	31(7.4)	21(2.0)	T3(3.0)	13(14.3)

* Multiple choice question

The result in Table 2.b showed that at preintervention (baseline phase), daily latrine cleaning
practice was poor, just among 61 (8.2%) respondents
which was later improved at post-intervention where 252
(33.9%) respondents affirmed cleaning their toilets daily.
At baseline, most of the respondents 491 (66.0%) had
once defaecated in the open in the past one year before
the time this study was conducted which was reduced at
post-intervention where only 49 (6.6%) reported to still
have defaecated openly within the period of intervention.
Open defaecation was more 28 (3.8%) in Biase Local
Government Area and least 6 (0.8%) in Yakurr Local
Government Area. At baseline, majority 308 (41.4%) of
respondents who had once open defaecated could not

remember the last time they ever openly defaecated but at post-intervention, only 12 (1.6%) could report open defaecation within the months of intervention.

The main source of drinking water for households was bottle/sachet water 467 (62.8%) at preand post-intervention. At pre-intervention, treatment of water before consumption was poor, where only 99 (13.3%) respondents admitted doing so but later improved to 681 (91.5%) after intervention. The major treatment options were boiling 41 (5.5%) and sedimentation 33 (4.4%) at baseline while the number increased to 222 (29.8%) and 272 (36.6%) at post-intervention. The major reason for not treating water was

300 (40.3%) due to high cost of treatment at baseline while after intervention, only 28(3.8%) respondents reported not to be knowledgeable in water treatment.

Across LGAs, few respondents 131 (17.6%) in Abi have the perception that the water source is safe and as such do not need treatment (Table 2.b).

Table 2.b: Toilet hygiene, drinking water source and treatment

Table 2.b: Toilet hygiene, drinking water source and treatment								
Variable	Baseline da		T			ention data	T	I
	Abi	Biase	Yakurr	Total	Abi	Biase	Yakurr	Total
Latrine cleaning practice								
Daily	32(4.3)	13(1.7)	16(2.2)	61(8.2)	80(10.8)	78(10.5)	94(12.6)	252(33.9)
Weekly	42(5.6)	41(5.5)	38(5.1)	121(16.3)	61(8.2)	63(8.5)	51(6.9)	175(23.5)
2-3 times a month	61(8.2)	53(7.1)	49(6.6)	163(21.9)	42(5.6)	53(7.1)	49(6.6)	144(19.4)
Once a month	80(10.8)	63(8.5)	51(6.9)	194(26.1)	33(4.4)	41(5.5)	38(5.1)	112(15.1)
Rarely	33(4.4)	78(10.5)	94(12.6)	205(27.6)	32(4.3)	13(1.7)	16(2.2)	61(8.2)
Defaecated in the open in								
the past 1 year					¥	¥	¥	¥
Defaecated openly	201(27.0)	184(24.7)	106(14.2)	491(66.0)	15(2.0)	28(3.8)	6(0.8)	49(6.6)
Never defaecated openly past	47(6.3)	64(8.6)	142(19.1)	253(34.0)	233(31.3)	220(29.6)	242(32.5)	695(93.4)
1 year								
Last time defaecation in the								
open (n=491)								
Today	12(1.6)	5(0.7)	10(1.3)	27(3.6)	1(0.1)	5(0.7)	0(0.0)	6(0.8)
Yesterday	13(1.7)	19(2.6)	6(0.8)	38(5.1)	3(0.4)	3(0.4)	2(0.2)	8(1.1)
Within this week	21(2.8)	21(2.8)	13(1.7)	55(7.4)	2(0.2)	2(0.2)	1(0.1)	5(0.7)
Within this month	41(5.5)	51(6.9)	34(4.6)	126(16.9)	4(0.5)	5(0.7)	3(0.4)	12(1.6)
Few months ago	80(10.8)	40(5.4)	38(5.1)	158(21.2)	41(5.5)	51(6.9)	38(5.1)	130(17.5)
Can't remember (Forgot)	167(22.4)	136(18.3)	5(0.7)	308(41.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
*Main source of drinking								
water for household								
members								
Piped line	32(4.3)	24(3.2)	25(3.4)	81(10.9)	32(4.3)	24(3.2)	25(3.4)	81(10.9)
Hand pump/borehole	80(10.8)	35(4.7)	64(8.6)	179(24.1)	80(10.8)	35(4.7)	64(8.6)	179(24.1)
Open dug well	40(5.4)	12(1.6)	12(1.6)	64(8.6)	40(5.4)	12(1.6)	12(1.6)	64(8.6)
Spring	13(1.7)	15(2.0)	10(1.3)	38(5.1)	13(1.7)	15(2.0)	10(1.3)	38(5.1)
Stream/River	81(10.9)	76(10.2)	83(11.2)	240(32.3)	81(10.9)	76(10.2)	83(11.2)	240(32.3)
Rain water	106(14.2)	91(12.2)	76(10.2)	273(36.7)	106(14.2)	91(12.2)	76(10.2)	273(36.7)
Bottle/Sachet water	141(19.0)	159(21.4)	167(22.4)	467(62.8)	141(19.0)	159(21.4)	167(22.4)	467(62.8)
Water vendor	61(8.2)	41(5.5)	27(3.6)	129(17.3)	61(8.2)	41(5.5)	27(3.6)	129(17.3)
Treating water before								
consumption								
Treats water before								
consumption	26(3.5)	31(4.2)	42(5.6)	99(13.3)	230(30.9)	220(29.6)	231()31.0	681(91.5)
Never treated water	222(29.8)	217(29.2)	206(27.7)	645(86.7)	18(2.4)	28(3.8)	17(2.3)	63(8.5)
Treatment options used (n								
= 99 & 681)	2(0.4)	2(0.0)	4(0.5)	0/1.0	21/2 0	20/2.5	21/2 0	(2 (0 2)
Water guard or chlorine	3(0.4)	2(0.3)	4(0.5)	9(1.2)	21(2.8)	20(2.7)	21(2.8)	62(8.3)
Cloth filtration	5(0.5)	4(0.5)	7(0.9)	16(2.2)	41(5.5)	32(4.3)	51(6.9)	124(16.7)
Sedimentation	6(0.8)	10(1.3)	17(2.3)	33(4.4)	81(10.9)	91(12.2)	100(13.4)	272(36.6)
Boiling	12(1.6)	15(2.0)	14(1.9)	41(5.5)	87(11.7)	76(10.2)	59(7.9)	222(29.8)
Reasons for not treating								
water (n = 645 & 63)	101/15	15/2 2	24/4 5	100/01 5	2(0.5)	2/0 /	2(0.5)	5 (0.6)
Water being safe	131(17.6)	17(2.3)	34(4.6)	182(24.5)	2(0.2)	3(0.4)	2(0.2)	7(0.9)
Expensive to treat	81(10.9)	92(12.4)	127(17.1)	300(40.3)	5(0.7)	6(0.8)	8(1.1)	19(2.6)
No knowledge to treat	7(0.9)	75(10.1)	20(2.7)	102(13.7)	10(1.3)	13(1.7)	5(0.7)	28(3.8)
Being used to water already	3(0.4)	33(4.4)	25(3.4)	61(8.2)	1(0.1)	6(0.8)	2(0.2)	9(1.2)

^{*} Multiple responses

Toilet Hygiene, Drinking Water Source and Treatment Using the Observational Check List

The results in Table 2.c at baseline showed that 381 (51.2%) households were found to have signs of faecal matter in their compounds but only 74 (9.9%) were households with no signs of faecal matter in their compounds whereas after the intervention, the results at

post-intervention showed that the signs of faecal matter was reduced to 35 (4.7%) and majority 678 (91.1%) of the compounds were devoid of signs of faecal matter (Table 2.c).

The baseline data showed that toilets were present in 260 (34.9%) households while at post-

[¥]Assessed for last one month on open defaecation

intervention phase, ownership of toilets increased to 469 (63.0%). At the baseline, of the 260 (34.9%) who had toilets, 90 (12.1%) had it outside the compounds, followed by 80 (10.8%) who had their toilet outside the house while only 20 (2.7%) had it in their rooms whereas after the intervention, of the 469 (63.0%), the number of households who built toilets in their rooms increased 192 (25.8%). Pit latrines with slap were predominant 90 (12.1%) at baseline while at post intervention it increased to 240 (32.3%). At baseline only 99 (13.3%) toilets had smooth cleanable floor while at post intervention 381 (51.2%) had cleanable floor. At baseline data, 272 (36.6%) of the toilets were observed to have no water source but at post intervention, it reduced to 33 (4.4%). There were toilet holes without slaps 300(40.3%) at preintervention but was later improved upon at postintervention to 182 (24.5%) toilets with closed devices. Fewer toilets 99 (13.3%) were found with no sign of excreta around at baseline which later got better 381 (51.2%) at post-intervention. Hand washing practice was

poor 20 (2.7%) on site at baseline which later was improved 192 (25.8%) after the intervention. The most prevalent water source at baseline 90 (12.1%) and post-intervention 240 (32.3%) was packaged/delivered water (Table 2.c).

Bacteriological Quality of Natural Water Sources during Pre- and Post- CLTS Intervention in the Study Area

A total of 24 natural water sources were assessed. Samples collected at baseline showed that out of the 24 samples, 12 (50.0%) sources had total and faecal coliform count that were Too Numerous to Count (TNTC). Still at baseline data, all the natural water sources had very high total and faecal coliform counts. Though the total and faecal coliform counts reduced at post-intervention phase, all the 24 natural water sources still had total and faecal coliform counts above the safe limits recommended by World Health Organization and the Nigerian Drinking Water Quality Standard (Table 3).

Table 2.c: Toilet hygiene, drinking water source and treatment using the observational checklist

Variable Variable	Baseline data			Post-intervention data				
	Abi	Biase	Yakurr	Total	Abi	Biase	Yakurr	Total
OD (Signs of faecal matter)								
In the house	10(1.3)	13(1.7)	14(1.9)	37(5.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Near the household (in the	165(22.2)	176(23.7)	40(5.4)	381(51.2)	13(1.7)	17(2.3)	5(0.7)	35(4.7)
yard)			, ,	, ,				
Outside the household	70(9.4)	60(8.1)	112(15.1)	252(33.9)	13(1.7)	15(2.0)	3(0.4)	31(4.2)
None	3(0.4)	1(0.1)	82(11.0)	74(9.9)	222(29.8)	216(29.0)	240(32.3)	678(91.1)
Presence/availability of a								
toilet								
Presence of toilet\	96(12.9)	60(8.1)	104(14.0)	260(34.9)	103(13.8)	165(22.2)	201(27.0)	469(63.0)
Absence of toilet	152(20.4)	188(25.3)	144(19.4)	484(65.1)	106(14.2)	105(14.1)	64(8.6)	275(37.0)
Toilet location								
In the room (self-contained)	11(1.5)	6(0.8)	3(0.4)	20(2.7)	55(7.4)	92(12.4)	45(6.0)	192(25.8)
In the house	22(3.0)	23(3.1)	25(3.4)	70(9.4)	28(3.8)	30(4.0)	31(4.2)	89(12.0)
Outside the house	49(6.6)	10(1.3)	21(2.8)	80(10.8)	18(0.2)	30(2.7)	53(7.1)	101(13.6)
Outside the yard	14(1.9)	21(2.8)	55(7.4)	90(12.1)	2(0.5)	13(0.7)	72(9.7)	87(11.7)
Toilet type								
Flush/pour flush to piped	21(2.8)	10(1.3)	49(6.6)	80(10.8)	30(4.0)	28(3.8)	31(4.2)	89(12.0)
sewer system								
Septic tanks	3(0.4)	11(1.5)	6(0.8)	20(2.7)	2(0.5)	13(0.7)	72(9.7)	87(11.7)
Ventilated improved pit	6(0.8)	3(0.4)	11(1.5)	20(2.7)	40(5.4)	12(1.6)	12(1.6)	64(8.6)
latrines								
Composting toilets	22(3.0)	23(3.1)	25(3.4)	70(9.4)	13(1.7)	15(2.0)	10(1.3)	38(5.1)
Pit latrines with slabs	49(6.6)	21(2.8)	55(7.4)	90(12.1)	81(10.9)	76(10.2)	83(11.2)	240(32.3)
Pit latrines without	11(1.5)	6(0.8)	3(0.4)	20(2.7)	106(14.2)	91(12.2)	76(10.2)	273(36.6)
slap/platform								
Hanging latrine	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Bucket latrine	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Toilet structure								
Smooth cleanable floor	26(3.5)	31(4.2)	42(5.6)	99(13.3)	130(17.5)	120(16.1)	131(17.6)	381(51.2)
Slab (in good condition)	22(3.0)	17(2.3)	20(2.7)	59(7.9)	50(6.7)	52(7.0)	59(7.9)	161(21.6)
Wall	13(1.7)	41(5.5)	25(3.4)	79(10.6)	21(2.8)	51(6.9)	63(8.5)	135(18.1)
Roof	34(4.6)	54(7.3)	45(6.0)	133(17.9)	49(6.6)	70(9.4)	69(9.3)	188(25.3)
Frame (for roofing structure)	2(0.3)	0(0.0)	0(0.0)	2(0.3)	1(0.1)	2(0.3)	2(0.3)	5(0.7)
Door (lockable)	28(3.8)	56(7.5)	35(4.7)	119(16.0)	40(5.4)	73(9.9)	81(10.9)	194(26.1)
Toilet in use		20/25						
Excreta inside the toilet	2(2.8)	20(2.7)	21(2.8)	62(8.3)	3(0.4)	2(0.3)	4(0.5)	9(1.2)
(latrine)	41/5 5	22/4.23	#1/c 0\	104/11=	5.0.5	4/0.5	7(0.6)	1.600.00
Water source available	41(5.5)	32(4.3)	51(6.9)	124(16.7)	5(0.5)	4(0.5)	7(0.9)	16(2.2)
Water source not available	81(10.9)	91(12.2)	100(13.4)	272(36.6)	6(0.8)	10(1.3)	17(2.3)	33(4.4)

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Toilets' defaecation hole								
Presence of closing device	2(0.2)	3(0.4)	2(0.2)	7(0.9)	131(17.6)	17(2.3)	34(4.6)	182(24.5)
Absence of closing device	81(10.9)	92(12.4)	127(17.1)	300(40.3)	5(0.7)	6(0.8)	8(1.1)	19(2.6)
Cleanliness of the toilet								
No signs of excreta around	26(3.5)	31(4.2)	42(5.6)	99(13.3)	130(17.5)	120(16.1)	131(17.6)	381(51.2)
No bad smell	22(3.0)	17(2.3)	20(2.7)	59(7.9)	50(6.7)	52(7.0)	59(7.9)	161(21.6)
No contact to animals/flies	13(1.7)	41(5.5)	25(3.4)	79(10.6)	21(2.8)	51(6.9)	63(8.5)	135(18.1)
Faeces disposal site								
In situ	3(0.4)	2(0.3)	4(0.5)	9(1.2)	23(3.1)	13(1.7)	19(2.6)	55(7.4)
Septic tank	5(0.5)	4(0.5)	7(0.9)	16(2.2)	10(1.3)	12(1.6)	16(2.2)	38(5.1)
Emptying (through company)	6(0.8)	10(1.3)	17(2.3)	33(4.4)	11(1.5)	20(2.7)	45(6.0)	76(10.2)
No emptying	213(28.6)	72(9.7)	68(9.1)	353(47.4)	43(5.8)	40(5.4)	12(1.6)	95(12.8)
Ditch	23(3.1)	32(4.3)	41(5.5)	96(12.9)	23(3.1)	23(3.1)	11(1.5)	57(7.7)
Closed pipe from toilet	13(1.7)	15(2.0)	19(2.6)	47(6.3)	12(1.6)	11(1.5)	4(0.5)	27(3.6)
In drains/gutter	46(6.2)	23(3.1)	71(9.5)	140(18.8)	9(1.2)	6(0.8)	2(0.3)	17(2.3)
Hand-washing practice								
Presence of HW practice on	11(1.5)	6(0.8)	3(0.4)	20(2.7)	55(7.4)	92(12.4)	45(6.0)	192(25.8)
site								
Water available	22(3.0)	23(3.1)	25(3.4)	70(9.4)	28(3.8)	30(4.0)	31(4.2)	89(12.0)
Soap available	49(6.6)	10(1.3)	21(2.8)	80(10.8)	18(0.2)	30(2.7)	53(7.1)	101(13.6)
Station designated for HW	14(1.9)	21(2.8)	55(7.4)	90(12.1)	2(0.5)	13(0.7)	72(9.7)	87(11.7)
Water source								
Piped water	21(2.8)	10(1.3)	49(6.6)	80(10.8)	30(4.0)	28(3.8)	31(4.2)	89(12.0)
Boreholes/tube wells	3(0.4)	11(1.5)	6(0.8)	20(2.7)	2(0.5)	13(0.7)	72(9.7)	87(11.7)
Protected dug wells	6(0.8)	3(0.4)	11(1.5)	20(2.7)	40(5.4)	12(1.6)	12(1.6)	64(8.6)
Protected springs	22(3.0)	23(3.1)	25(3.4)	70(9.4)	13(1.7)	15(2.0)	10(1.3)	38(5.1)
Packaged/delivered water	49(6.6)	21(2.8)	55(7.4)	90(12.1)	81(10.9)	76(10.2)	83(11.2)	240(32.3)
Unprotected dug well/spring	11(1.5)	6(0.8)	3(0.4)	20(2.7)	106(14.2)	91(12.2)	76(10.2)	273(36.6)
River, dam, lack, pond,	6(2.8)	11(1.5)	10(1.3)	27(3.6)	6(2.8)	11(1.5)	10(1.3)	27(3.6)
stream, canal								
Unprotected river, dam, lake,	20(2.7)	21(2.8)	22(3.0)	63(8.5)	22(3.0)	20(2.7)	20(2.7)	62(8.4)
etc.								
Collection time								
Over 30 minutes	26(3.5)	31(4.2)	42(5.6)	99(13.3)	130(17.5)	120(16.1)	131(17.6)	381(51.2)
Under 30 minutes	22(3.0)	17(2.3)	20(2.7)	59(7.9)	50(6.7)	52(7.0)	59(7.9)	161(21.6)
On-site	13(1.7)	41(5.5)	25(3.4)	79(10.6)	21(2.8)	51(6.9)	63(8.5)	135(18.1)

Table 3: Bacteriological quality of natural water sources

S/n	Natural water	Baseline data	iological quality of hate	Post-intervention data			
	sources	Total coliform count	Faecal coliform count	Total coliform count	Faecal coliform count		
		(cfu/100ml)	(cfu/100ml)	(cfu/100ml)	(cfu/100ml)		
1	Anong stream	131	99	87	52		
2	Akpoha stream	126	103	90	78		
3	Imina stream	116	90	96	62		
4	Ivone creek	TNTC	TNTC	135	121		
5	Igbor pond	135	109	99	70		
6	Itigeve creek	TNTC	TNTC	142	135		
7	Ngarabe stream	110	92	88	52		
8	Akpanku stream	142	127	101	85		
9	Iwuru pond	TNTC	TNTC	140	96		
10	Ehom stream	123	116	99	67		
11	Okurike creek	TNTC	TNTC	103	52		
12	Ibenabang	TNTC	TNTC	93	71		
	stream						
13	Akpet central	125	113	64	51		
	stream						
14	Umai creek	TNTC	TNTC	102	60		
15	Etana stream	TNTC	TNTC	85	41		
16	Ipene stream	TNTC	TNTC	69	48		
17	Ugom stream	TNTC	TNTC	107	71		
18	Ijiman pond	126	103	34	32		
19	Afrekpe creek	TNTC	TNTC	109	61		
20	Levate pond	TNTC	TNTC	57	40		
21	Egbizum stream	128	111	56	33		

22	Lebolkom pond	112	98	112	98
23	Yenon stream	TNTC	TNTC	51	29
24	Ikpakapit stream	131	105	62	31
	WHO safe limits	0	0	0	0
	NDWQS limits	0	0	0	0

TNTC - Too Numerous to Count

DISCUSSION

Latrine/Toilet Ownership and Use by Respondents

The finding in this part of the study showed that at pre-intervention, more than half 425 (57.1%) of the respondents had no latrine/toilets and this was highest 185 (24.9%) in Biase and least 63 (8.5%) in Yakurr L.G.A.s while after the intervention, the postintervention data showed that latrine/toilet ownership increased by 43.4% giving a total of 642 (86.3%) latrine/toilet ownership and was highest 224 (30.1%) in Yakurr and least 207 (27.8%) in Abi L.G.A.s respectively. This is in line with the finding by Harter et al., (2018) whose finding indicated that increasing latrine ownership happened concurrently with increased dissemination of CLTS-related information, with the greatest rise occurring in the group that engaged in CLTS. The study also showed that the most owned type of latrine/toilet was pit latrine without slap 114 (15.3%) at baseline while at post-intervention, pit latrine without slap was still found to be the most owned latrine/toilet 194 (26.1%). The least 66 (8.9%) at baseline and 44 (5.9%) at post-intervention were pour flush and latrine draining to canal/stream/river respectively. At baseline, use of nearby bush/gutter/stream was high 492 (66.1%) whose responses were same after intervention perhaps because the question was to access those who had ever involved in the act of using nearby bush/gutter/stream for defaecation. This is in line with Tessema (2017) who reported that 22% of the residents owned toilets following CLTS in the study area.

Most of respondents 432 (58.1%) had their toilets 50 metres away from their houses (pre- and postintervention). More than 7 members 304 (40.9%) share latrines (pre- and post-intervention). Majority 310 (41.7%) defaecate in public latrines in the absence of self-owned latrines (pre- and post-intervention). Most 110 (14.8%) of the reasons for non-ownership of latrines is high cost owning one (pre- and post-intervention). Disposal of baby/infants' faeces was mostly 235 (31.6) done by burying at baseline but was later improved at post-intervention by disposal of baby/infants' faeces in toilet 273 (36.7%). At baseline, hand washing after the use of toilet was poor 158 (21.2%) for those who do that always while it was improved at post-intervention where those who always practice hand washing after use of toilet were 296 (39.8%). This finding is supported by Gebremariam et al., (2018) who reported that distance between the latrine and the residence was associated with OD practice. Daily latrine cleaning practice was poor 61 (8.2%) at pre-intervention which was later improved at

post-intervention where 252 (33.9%) reported to be cleaning their toilet daily.

The main source of drinking water for households was bottle/sachet water 467 (62.8%) at preand post-intervention. At pre-intervention, treatment of water before consumption was poor 99 (13.3%) but later improved to 681 (91.5%) after intervention. The major treatment options were boiling 41 (5.5%) and sedimentation 33 (4.4%) at baseline while the number increased to 222 (29.8%) and 272 (36.6%) at postintervention. The major reason for not treating water was 300 (40.3%) due to high cost of treatment at baseline while after intervention, 28 (3.8%) reported not to be knowledgeable in water treatment. Across LGAs, few persons 131 (17.6%) in Abi have the perception that the water source is safe and as such do not need treatment. The test of hypothesis at both pre- and post-intervention was significant (P = 0.001). It was therefore concluded that there is a statistically significant association between access to toilet and the reduction of open defaecation practices among residents following the CLTS intervention.

Using the observational checklist, the results at baseline showed that 381 (51.2%) households were found to have signs of faecal matter in the yard but only 74 (9.9%) where households with none signs for faecal matter whereas after the intervention, the results at postintervention showed that the signs of faecal matter was reduced to 35 (4.7%) but majority 678 (91.1%) were devoid of signs of faecal matter within the yard. The baseline data showed that toilets were present in only 260 (34.9%) households while at post-intervention phase, ownership of toilets increased to 469 (63.0%). At the baseline data, of the 260 (34.9%) who had toilets, 90 (12.1%) had it outside the yard, followed by 80 (10.8%) who had their toilets outside the house while only 20 (2.7%) had it in their rooms whereas after the intervention, of the 469 (63.0%), the number of households who built toilets in their rooms increased to 192 (25.8%).

This finding also showed that pit latrines with slap were predominant 90 (12.1%) at baseline while at post-intervention it increased to 240 (32.3%). At baseline only 99 (13.3%) toilets had smooth cleanable floor while at post intervention 381 (51.2%) had cleanable floor. At baseline data, 272 (36.6%) of the toilets were observed to have no water source but at post-intervention, it reduced to 33 (4.4%). There were toilet holes without slap 300 (40.3%) at pre-intervention but was later improved upon at post-intervention to 182 (24.5%)

toilets with closed devices. Fewer toilets 99 (13.3%) were found with no sign of excreta around at baseline which later got better 381 (51.2%) at post-intervention. Hand washing practice was poor 20 (2.7%) on site at baseline which later was improved 192 (25.8%) after the intervention. The most prevalent water source at baseline 90 (12.1%) and post-intervention 240 (32.3%) was packaged/delivered water. The test of hypothesis at both pre- (p = 0.002) and post-intervention (p = 0.001) was significant (P < 0.05). It was therefore concluded that there is a statistically significant association between access to improved water sources and the reduction of sanitation-related diseases among residents following the CLTS intervention.

Bacteriological Quality of Natural Water Sources during Pre- and Post- CLTS Intervention

A total of 24 natural water sources were assessed. Samples collected at baseline showed that out of the 24 samples, 12 (50.0%) sources had total and faecal coliform count that were Too Numerous To Count (TNTC). Still at baseline data, all the natural water sources had very high total and faecal coliforms. Though the total and faecal coliforms count reduced at postintervention phase, all the 24 natural water sources still have coliform counts above the safe limits recommended by World Health Organization. This finding is in tandem with Babb et al., (2018), in Kenya's Nyando District, where it was reported that intervention's effectiveness in open defaecation-free villages was being jeopardized by faecal pollution of their water sources and the proportion of Escherichia coli in open defaecation-free villages' source of water supply was greater than in non-open defaecation-free communities (i.e. 76.7% against 60%). In consonance with this present finding is the conclusion by Venkataramanan et al., (2018) that even at pre- and post-intervention, there were still coliforms found in water sources of households used in the study. However, there was rather a reduction in the counts in the water sources after the intervention.

CONCLUSION

Open defaecation practice remains a serious public health problem in low and middle-income countries, including Nigeria. The drive to protect human health and improve environmental sanitation would essentially require ending open defaecation practice. In line with the findings of this study, it was revealed that community-led total sanitation (CLTS) has demonstrated significant impact in reducing open defaecation and mitigating its harmful health implications in the study area. By empowering communities to take ownership of sanitation, CLTS intervention was effective in increasing knowledge level on open defaecation practice, changing wrong perceptions about open defaecation, improving toilet ownership and maintenance, improving access to sanitation facilities, reduced open defaecation practices significantly, enhanced community-led initiatives for sustainability, decreased incidence of sanitation-related diseases and fostered improved sanitation and hygiene practices.

Water quality control within the permissible limits have become a top priority in Cross River State. This is significantly due to the outbreak of sanitationrelated diseases affecting mostly the rural populations. The World Health Organization expects that water meant for household is free from pathogenic microorganisms or any form of contamination from source to point of use. In this study, the bacteriological analysis showed that total coliform and faecal coliform counts for all water samples exceeds World Health Organisation (WHO) and Nigeria Standard for Drinking Water Quality (NSDWQ) limits. Hence, to improve access to potable water and water quality, open defaecation must be completely eradicated and effective triad synergy between the host communities, implementing partners (such as NGOs), and the government should be established.

Acknowledgment

The authors appreciate the academic staff of the Department of Public Health, Federal University of Technology, Owerri and the University of Calabar for their contributions towards the research work and also appreciate co-authors for their commitment during the research.

Grant Support Details: The research was part of my PhD dissertation, fully funded and carried out by Inah, Simon Alain.

Conflict of Interest

The authors declare that there is not any conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

Life Science Reporting: No life science threat was practiced in this research.

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