

**EPIDEMIOLOGICAL STUDIES OF WATERBORNE DISEASES IN RELATION TO
BACTERIOLOGICAL QUALITY OF WATER**

Abstract

Aim of the study: Waterborne diseases are global burden with increase in number of cases more especially in rural areas of developing countries. We investigated the epidemiological distribution of waterborne diseases and bacteriological quality of water in Bodinga Sokoto Nigeria.

Research design: Research Design

The study used a cross-sectional design and determined the prevalence of some selected waterborne diseases and sanitary inspection. An experimental design was used for determination of bacterial pollution in some water sources.

Place and duration: The study was conducted at the General Hospital Bodinga and Department of Microbiology Sokoto state University within the period of one year.

Methods: A retrospective data of health records were collected from out-patient register in Bodinga General Hospital, covered a period of three years from January to December (2015 -2017). A number of samples of water were collected from different sources in Bodinga, Danchadi and Takatuku and were analyzed using standard method.

Results: We found the most common waterborne diseases in the area are dysentery, 517(40.7%) typhoid 375(29.5%), gastroenteritis 202(15.9%) and diarrhea 105(8.3%), while skin infection and cholera account for 36(2.8%) each. We observed that the diseases are widely distributed in rainy season with high occurrence of 732(57.59%) cases than dry season having 539(42.41%) cases. Male are more prone to diseases with 706 cases than female having 565 cases and 25-above years as well as Children below the age of 5 are more vulnerable to diseases with occurrence of 481 and 331 respectively.

Conclusion: This study suggests a possible strong relationship between waterborne diseases and poor water quality which contributed to the spread of diseases in the study area.

Key words: Epidemiological studies; E. coli; Typhoid; Gastroenteritis; Skin infections; Borehole, Shallow well; Water; quality; Bodinga

1.0 INTRODUCTION

Water is important means of sustenance that are needed by all forms of life. Many diseases associated with water are occurred as result of ingestion of water. Globally, at least 2 billion people use a drinking water source contaminated with of human or animal faeces (WHO, 2018). Most of the population of people in developing countries particularly in rural live in extreme conditions of poverty and poor sanitation services in public places as well as

40 inadequate water supply and poor hygiene including hospitals, health centers and schools
41 (WHO, 2018). Statistically, World Health Organization reported at least 884 million people do
42 not have access to basic drinking water and almost 159 million people are dependent on
43 rivers and lakes with almost 423 million people taking water from unprotected springs and
44 wells (WHO, 2017).

45 Waterborne disease outbreaks are mainly occurred due to technological failures or failure to
46 treat the water properly (Craun and Calderon, 2006). Waterborne diseases can be
47 transmitted through faecal oral route and direct contact. Some parasites are capable of
48 penetrating intact skin and cause severe infection such as skin infection. In developed
49 countries, waterborne disease is no longer considered a constant threat (Moreira *et al.*,
50 2005).

51 In context to developing countries like Nigeria microbial quality of water is a downside due to
52 lack of essential state of art facilities for treatment of water and financial allocations.
53 However access to safe water, particularly to dwellers in rural, settlement and villages in
54 remote areas, is difficult at intervals a brief distance. Contaminations of drinking water with
55 pathogens have additionally being reported in several towns in Nigeria (Ibrahim *et al.*, 2000;
56 Adekunle *et al.*, 2007; Biu *et al.*, 2009).

57
58 In addition, people consuming unfit drinking water become infected with pathogens if a
59 proper measure to eliminate the pollutant in the water is not taken. In this work, we found
60 that most of the people lived in rural areas of Bodinga observe open laxation which can
61 result in the contamination of sources as the fecal matters washed away during the rainy
62 season. Lack of knowledge, sanitary healthful facilities, and sensible hygienic practice
63 contribute meaningfully to unfold of wbd within the area. We found out the presence of
64 animals within a short distance of water sources, for instance, in the shallow well animal are
65 available while people are fetching water for their daily activities.

66 It has been reported the grazing of animal and pasteurization nearby water sources
67 significantly affects the quality water (Hubbard *et al.* 2003; and Yang *et al.*, 2004 and Nafiu
68 and Anandapandian, 2016) and may lead to the entry of pathogens into the water bodies.
69 Gastroenteritis is an abdominal infection associated with some similar symptoms as diarrhea
70 which has heterogeneous causative agents.

71 The outbreaks of enteric disease due to water have occurred both when public drinking
72 water supplies were contaminated with surface water and when surface waters
73 contaminated with enteric pathogens have been used for recreational purpose (Johnson *et al.*
74 *et al.*, 2003). Drinking water quality can be assessed by detecting indicator organisms which
75 their presence indicate contamination with biological origin and thus, present potential health
76 impact and risk associated with the consumption of unfit water. *E coli* is the most reliable
77 indicator organisms of water pollution which are considered as the organisms of choice to
78 indicate recent fecal contamination in drinking water (Leclerc *et al.* 2001; Payment *et al.*
79 2003; Wade *et al.* 2003; Tallon *et al.*,2005; Verhille, 2013). Some strains of *E. coli* are non-
80 pathogenic while other strains are found to be pathogenic which provide a clue on the
81 presences of the enteric pathogen in water (Tallon *et al.* 2005), but at present, *E. coli*
82 appears to provide the best bacterial indicators of fecal contamination in drinking water
83 (WHO 2008). This is based on the following: (i) the occurrence of thermotolerant (fecal)
84 coliforms in temperate environments as compared to the rare incidence of *E. coli*; (ii) the
85 presence of *E. coli* in human and animal waste and customarily not elsewhere within the
86 environment; and (iii) the supply of cheap, fast, sensitive, specific and easier check
87 strategies to sight *E. coli*. Therefore, *E. coli* is the best and commonest microbial indicator
88 available to date to inform public health risks associated with the consumption of
89 contaminated drinking water (Staradumskyte & Paulauskas 2012; Odonkor & Ampofo,
90 2013).

91
92 According to UN Environment Programme (UNEP), 300 million people in Africa still do not
93 have reasonable access to safe drinking water and nearly 230 million people defecate in the
94 open (Vidal 2012). Waterborne diseases are caused by several pathogenic microorganisms

95 that include bacteria, viruses, protozoan and helminthes. This is usually occurred as a result
96 of poorly treated drinking water and waste water or natural disaster like flooding and
97 environmental pollution (Adeyika *et al.*, 2014).

98 The pathogen load in the water body from several contamination sources varies strongly
99 with time, often due to the prevalence and incidence of the disease in the community. Under
100 epidemic conditions, pathogens are excreted from many more human or animal hosts than
101 under endemic conditions. An increased pathogen load, which enters the water source with
102 wastewater discharges or surface runoff, implies increased risk for waterborne infections
103 (Field and Samadpour 2007).

104
105 Furthermore, the current study geared toward investigation the epidemiological distribution
106 of waterborne diseases in relation to bacteriological quality of water in Bodinga town. Here,
107 we shed a light on bacterial waterborne diseases that are prevalent in the study area. These
108 cases had not been reported or documented properly within the study area in spite of studies
109 conducted by Raji and Ibrahim (2011) in two alternative areas of Sokoto, however the
110 researchers do not capture Bodinga. Seeable of the cases discovered throughout our
111 preliminary survey, it is necessary to investigate the occurrence of waterborne diseases
112 within the crony villages of Bodinga in order to create awareness and set an alarm to
113 responsible authorities.

114 **2.0 METHODOLOGY**

115 **2.1 Study Area**

116 The area of study is Bodinga Local government in Sokoto state, Nigeria. Its headquarters
117 are in the town of Bodinga. It has an area of 564 km² and a population of 175,406 at the
118 2006 census. The postal code of the area is 852. Bodinga town is 11 km away from Sokoto
119 town and it has limited rainfall from mid-May to October. It is also subjected to Sahara's
120 hamattan from November to March.

121 **2.2 Research Design**

122 The study used a cross-sectional design and determined the prevalence of some selected
123 waterborne diseases and sanitary inspection. An experimental design was used for
124 determination of bacterial pollution in some water sources.

125 **2.3 Determining Prevalence of Water Borne Diseases**

126 The method described by (Nafi'u *et al.*, 2018) was employed and determined the type and
127 frequency of distribution of water borne diseases in Bodinga town. Retrospective data of
128 medical records from out patient record register in Bodinga general hospital for complete
129 three years (36 months) from January to December (2017, 2016 and 2015) were reviewed to
130 identify common waterborne diseases in the study area in respect to the year, month, age,
131 gender and season. About 1271 cases were reviewed.

132 **2.4 Bacteriological analysis of water/Collection of water sample**

133 The samples were collected in three (3) different villages within the Bodinga local
134 government. The village includes Takatuku, Danchadi and Bodinga. In each of the
135 mentioned villages, we consider two water sources for samplings, from each villages making
136 a total of six different sources because they are frequently used by the inhabitant of the
137 areas. Additionally, the method described by Abdulkadir and Anandapandia (2016) with
138 some modifications was adopted for collection of water samples. In brief the water samples
139 for bacteriological analysis were collected in sterile bottles from protected boreholes and
140 shallow wells under sterile condition using labeled sterile glass bottles (250ml) and
141 transported to the to the microbiology laboratory of Sokoto state university in a cool box at
142 4°C for analysis.

143 At water sources, the cap of a 250 ml sterile bottle was removed aseptically. The bottles
144 were filled from the water outflow pipe at boreholes. At the shallow well the cap of 250 ml
145 sterile bottle was removed and tight with a clean rope, it was inserted inside the shallow well
146 filled with water and pulled out. About one inch of space was left at the top of full bottles. The
147 cap was replaced aseptically. The procedure was repeated throughout the period of sample
148 collection. The bacteriological indicator of water quality analyzed was *Escherichia coli* using
149 multiple tube fermentation techniques and estimation through most probable number method

150 using the standard method described by (Cheesbrough, 2006; APHA 2005). Detail
151 description of the method was presented in supplementary 1.

152 **2.5 Data Analysis**

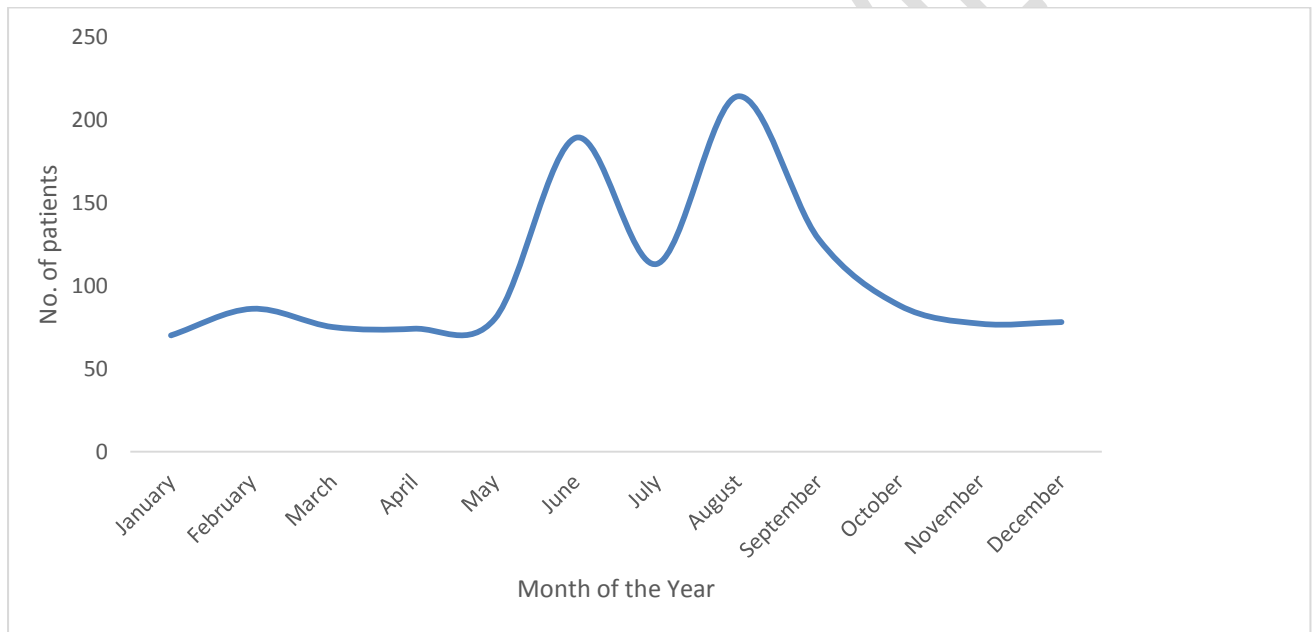
153 The data generated during the course of study were subjected to analysis using SPSS
154 (version 20). And Microsoft excel 2010. Descriptive statistics using mean and standard error
155 were used for the analysis of data. A simple graph and bar charts were also used for the
156 presentation of data concerning prevalence and distribution of waterborne diseases.
157 Inferential statistics of t-test, and ANOVA were used to test significance differences between
158 the variables.

159 **3.0 RESULTS**

161 **3.1 Prevalence and distribution of waterborne diseases in Bodinga Local government**

162 The prevalence and distribution of waterborne diseases in Bodinga town was conducted by
163 reviewing the out patients records from General Hospital Bodinga which covered a period of
164 2015 to 2017. About 1271 cases of waterborne diseases were investigated. The results for
165 distributions of waterborne diseases in Bodinga town in general are shown in (figure 1) and
166 details data of are also given in table 1 of supplementary sheet (S2).

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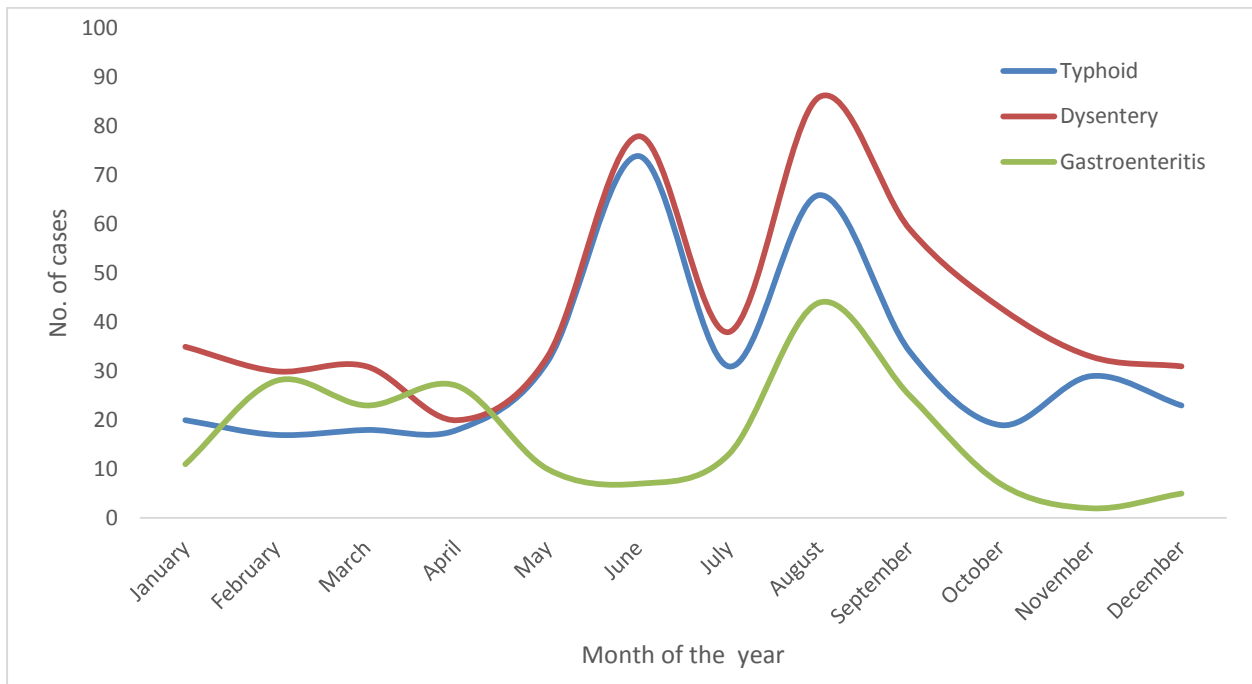
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170 **Figure 1: Spatial and temporal Distributions of diseases in Bodinga Local government area**

171
172 Figure 1 show how the diseases are distributed within the different months of the year in
173 Bodinga town. Generally, the month of August was observed with the highest variety of
174 diseases (214) June (189), September (127), and July (113) with the month of January
175 recording the bottom variety of cases. The fashion of the diseases is cyclic in nature, with
176 the first cycle moving to the right direction by increasing from January and peaking in June,
177 which falls down in July and next cycle begins. The second cycle increases from July to
178 October. The average number of the second cycle is 117 ± 52 cases which are higher than
179 98.14 ± 39.40 cases as in the first one. Although an independent t-test shows significant
180 difference (2.571 at $p = 0.05$), this pattern is extremely vital for planning in the health center
181 in terms of budgeting and allocation of human resources. Even though, most of these
182 diseases are preventable, awareness and prevention programs can be planned during
183 January – June as well as July –November window.

184 Dysentery 517(40.7%) typhoid 375(29.5%), and gastroenteritis 202 (15.9%) (Supplementary
185 3) are the highest most contributors of waterborne diseases in Bodinga local government
186 area. The spatial-tempo distributions of these diseases with time are shown in figure 2
187 below.

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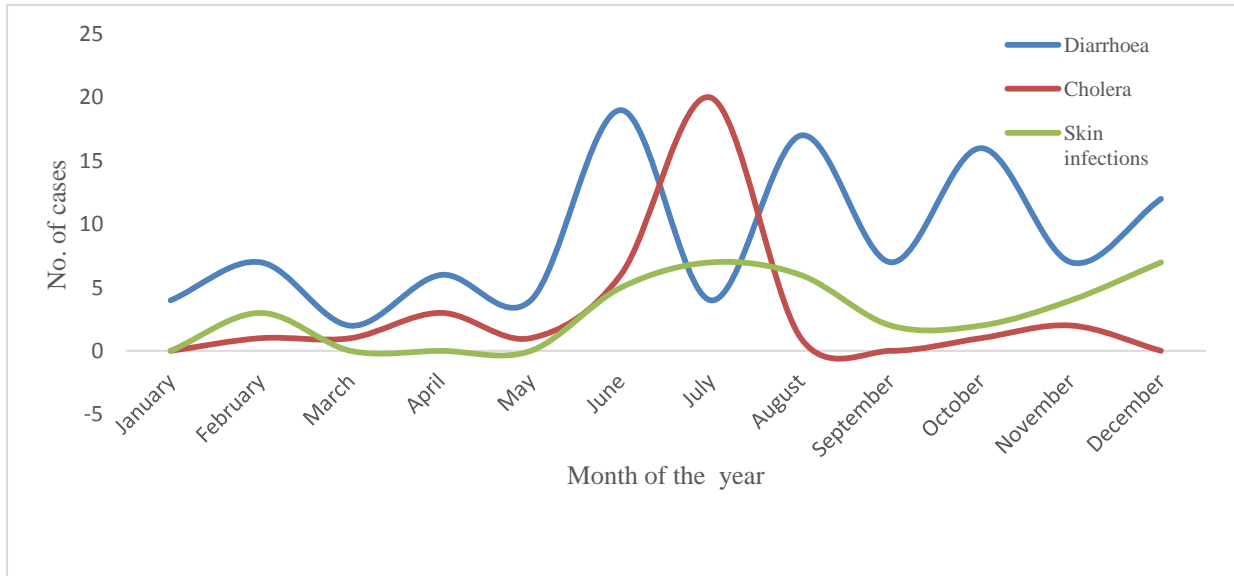


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194 **Figure 2: Spatial Distribution of typhoid, dysentery and gastroenteritis in Bodinga**
195 **Local government area**

196 Diarrhoea 105(8.3%), cholera and skin infections 36(2.8%) (Supplementary 3) are most
197 contagious diseases that fortuitously contribute least to the overall number of water borne
198 diseases in Bodinga local government area. These diseases can affect a number of the
199 population causing inflicting vital impacts within a short time. It is therefore important to
200 understand how these three occur within the year. The distributions of diarrhoea, cholera

201 and skin infection with time are shown in figure 3.



202

203 **Figure 3: Spatial Distribution of Diarrhoea, cholera and Skin infection in Bodinga**
 204 **Local government area**

205 **3.2 Seasonality Distribution of Waterborne diseases**

206 **Table 1: Seasonal distribution of water borne diseases**

Season	Waterborne diseases						Total
	Typhoid	Dysentery	Diarrhoeae	Cholera	Gastroenteritis	Skin infection	
Dry season	157	213	42	8	105	14	539
Wet season	218	304	63	28	97	22	732
Total	375	517	105	36	202	36	1271

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208 Seasonal patterns of waterborne diseases event suggests the hypotheses about how
 209 transmission of diseases occurs. Water serves as vehicle for transmission of diseases. The
 210 results represent seasonal characteristic pattern and variation in these diseases that might
 211 be because of flooding, washed away of soil and other alternative contaminant in to the
 212 water sources.

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214 **3.3 Gender classification of wbd**

215 The distribution pattern of wbd in respect to gender answered the developed analysis
 216 question how can variations of diseases occurred across the gender of the patients.

217 Statistically, an independent t- test show a significant difference between the gender of the
 218 patients and waterborne diseases.

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Table 2: Gender of the patient

	Waterborne diseases							Total
	Typhoid	Dysentery	Diarrhoeae	Cholera	Gastroenteritis	Skin infection		
Gender of the patient	Male	215	290	61	17	106	17	706
	Female	160	227	44	19	96	19	565
	Total	375	517	105	36	202	36	1271

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3.4 Secular Trends of WBD

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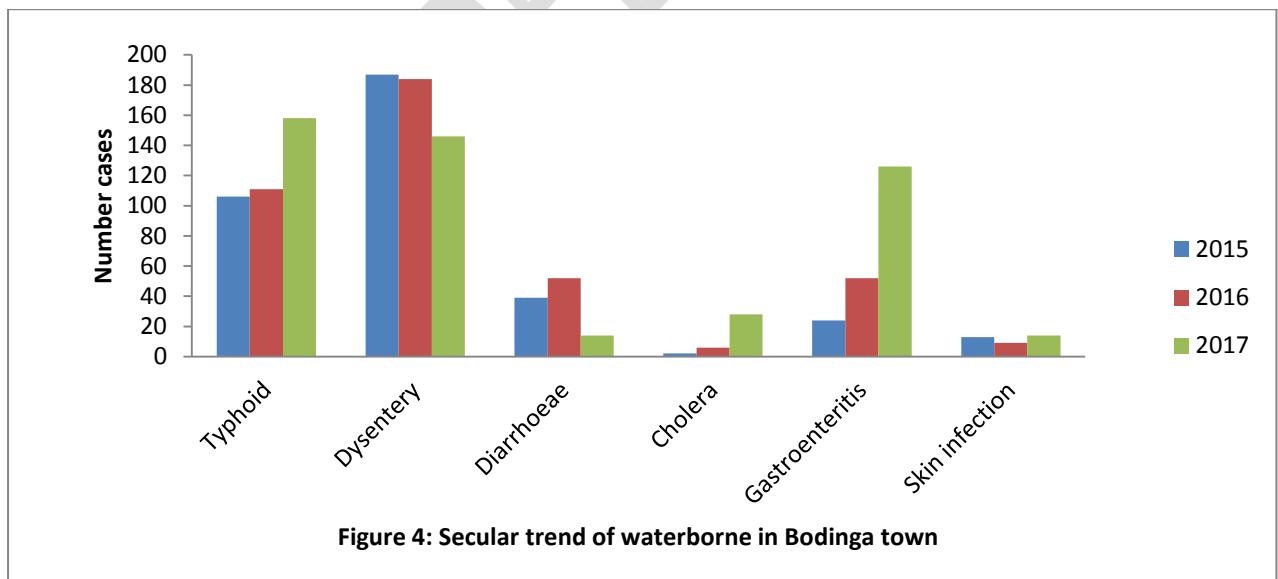
A secular trend in the occurrence of the diseases indicates that there is steady increasing of diseases for long-term in there near future. Year 2017 accounting highest prevalence of some diseases compared to other. The doable clarification might be because of increase in population and open defecation within the space which might contaminate water sources during rainfall. There is need for awareness program during the two windows as suggested earlier and policy decision to avoid recurrence of the diseases within the population.

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3.5 Age group distributions of WBD

236 The results of the prevalence of waterborne diseases is step with completely different age
 237 groups showed that, 25-above group has the very best prevalence in each diseases with
 238 481 (37.8%), groups followed by 0-4 with 331(26.0%), cases and 10-14 has 152(12.0%),
 239 while 5-9, 15-19 have the same prevalence with 99(7.8%) each, and the last group 20-24
 240 has 109 (8.6%), as shown above in table 3. The level of vulnerability of the patients i.e.
 241 Age and specific waterborne disease is also shown in table 3. It is evident that generally in
 242 all age groups, dysentery and typhoid were the foremost common disease. The age groups
 243 0-4years and > 25 years were additionally prone to all diseases with the exception seen in
 244 cholera where 0-4 recorded highest case. Further analyses of mean \pm SE have the
 245 subsequent values. The results of the different age groups of waterborne diseases disclosed
 246 the statistical significance differences between the age groups in table 3

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250 **Table 3: Age groups classification of waterborne diseases**

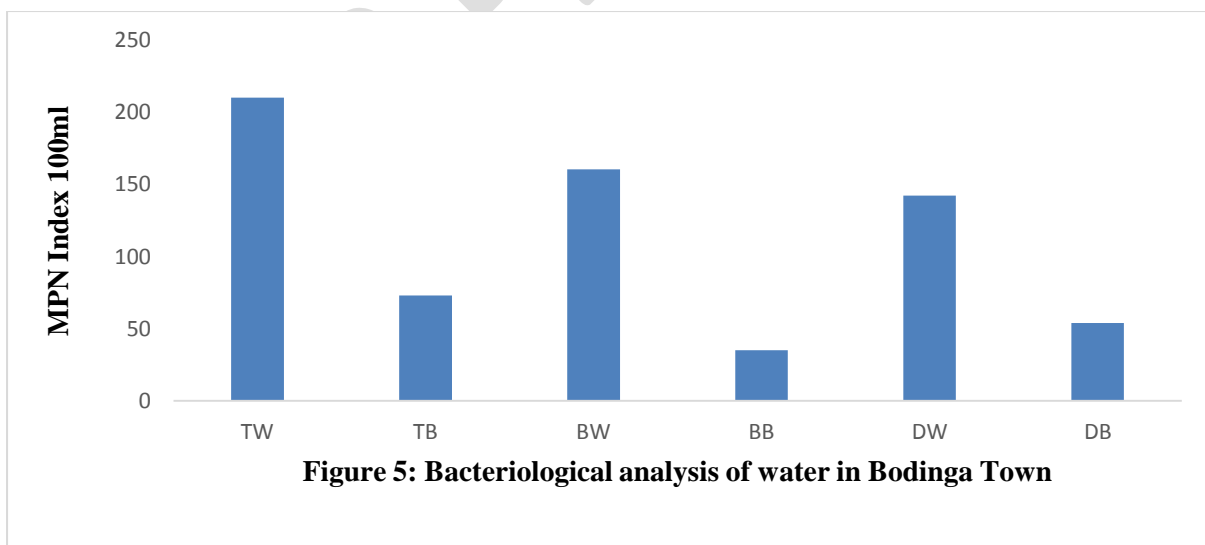
Age groups	Waterborne diseases						Total	Average \pm Std. Error
	Typhoid	Dysentery	Diarrhoea	Cholera	Gastroenteritis	Skin infection		
0-4	73	151	34	14	58	1	331	2.50 \pm .075
5-9	25	40	13	0	15	6	99	2.58 \pm .156
10-14	51	57	13	2	25	4	152	2.30 \pm .122
15-19	28	37	9	7	15	3	99	2.53 \pm .151
20-24	34	41	10	4	19	1	109	2.41 \pm .139
25-above	164	191	26	9	70	21	481	2.36 \pm .070
Total	375	517	105	36	202	36	1271	

251

252 **3.6 Bacteriological Analysis of water**

253 The analysis based on bacteriological quality of water, suggested the level of contamination
254 of the most reliable sources of drinking water in these communities revealed by the presence
255 of indicator organism. Thus, their presence in the drinking water generally indicate the
256 presence of pathogenic microorganisms in the water. The data presented in figure 5 is the
257 mean values of routinely samples analyzed from each sampling points. Table (S4)
258 summarizes the results of fecal contamination of drinking water sources analyzed in three
259 villages of Bodinga town (Bodinga Danchadi and Takatuku).

260 The number of *E.coli* detected in the all samples were found outside the limit conseled by
261 WHO and NCWR. The average number of indicator organisms in boreholes was 54.1 and
262 shallow well was 171.2 across all the three villages. Briefly, the average mean of fecal
263 contamination in Bodinga borehole was 35.1 and shallow well 160.7 as well as the average
264 highest contamination of boreholes and wells were recorded in Takatuku 210.4, 73.2 and
265 least in Danchadi 53.9, 142.5 (supplementary S4) showing an increased in fecal
266 contaminations of water sources in the areas. The analysis of variance revealed significance
267 difference for the concentration of *E.coli* in water (2.477 at $p = 0.005$).



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271 **Figure 5: Bacteriological analysis of water in Bodinga Town.** All the water samples
272 analyzed were in triplicate for each sampling point. The result is the average of the amount
273 of *E. coli* detected in the different water sources covered by the sampling points and each
274 bar is representing the sampling point (TW = Takatuku well, TB = Takatuku borehole, BB =
275 Bodinga borehole, BW = Bodinga well, DB = Danchadi borehole and DW = Danchadi well).

276

277 **Discussion**

278 Generally, the results of the present study for the distribution and variation of waterborne
279 diseases in Bodinga Local Government indicated that almost all of the waterborne diseases
280 are high in June and August. This is probably because of the intensity of rainfall in these
281 months which increases water percolation and runoff that may be carrying pollutants. Ejaz
282 (2011), reported waterborne disease was most rife throughout wet seasons.

283 The unhealthful hygienic conditions or poor environmental conditions around the drinking
284 water sources might contribute to high incidence of cases. The findings conjointly indicated
285 the month of August recording the highest quantity of cases followed by June while the
286 months of January and November have the lowest quantity of cases. The month of June and
287 August have high occurrence of cases tormented from waterborne diseases within the study
288 space.

289 The most frequent contributors of waterborne diseases in Bodinga local government are
290 dysentery, typhoid and gastroenteritis in Bodinga town. Similarly, the study of (Nwidi *et al.*,
291 2008), indicated typhoid fever, dysentery, cholera, and diarrhea, are the foremost reportable
292 waterborne disease in Ammassoma, Niger Delta, Nigeria. This is in line with the findings of
293 (Mohammad *et al.*, 2012) who found that there is a significant relationship between hygiene
294 and waterborne disease.

295 The patterns for diarrhea, skin infection and cholera diseases are similar to that in figure 3
296 showing dual cyclic pattern. This implies the intervention period suggested earlier still works
297 for interference and management of these diseases. Estimated cases of waterborne
298 diseases are 4.1% of the global burden with almost 1.8 million human deaths annually
299 during which 88% is attributed to unfit water supply, sanitation and poor personal hygiene
300 (WHO, 2017).

301 The results of these studies seem to point out that highest cases of the waterborne diseases
302 in Bodinga town occur during the wet season e.g. June – October. The results for
303 Diarrhoea, cholera and Skin infection are not exceptional of this observation with the pattern
304 quite different from other (Figure 3). These could be due to contamination of water sources.
305 Majority of the community living in Bodinga town used pit latrines it is more seemingly the
306 fecal material finds its way into water. Harper (2015) reported that runoff is capable of
307 transporting pathogens into water sources, increasing the risk of human exposure and
308 infection.

309

310 The seasonal event of WBD increases over the years as the rate of flooding increases.
311 Rainy season contributes greatly the spread of WBD in the area due to some factors that
312 increases the risk of diseases that embody agricultural runoff, washing away of fecal
313 materials into open and surface water sources. In our previous study we reported some
314 factors that increase the rate of diseases in rainy season these includes blocked drains,
315 increase in precipitation, flooding sewer and compromised system. Curriero *et al.* (2001)
316 reported floods can increase human susceptibility to pathogens due to spread of
317 contaminants by flood waters.

318 The high risk of WDB as a result of rainfall is an index of water pollution. Shallow well water
319 are contaminated as a result of sinking fecal matter which are carried by flood during a
320 heavy rainfall (Abdulkadir and Anandapandian, 2016).

321 Infection with waterborne pathogens has been shown to be higher during the wet season.
322 Thus, the high risk of water-borne diseases during the period of heavy rains is a key of
323 higher water pollution. Going by the finding of (Oguntoke *et al.*, 2009) cholera cases in
324 Ibadan were more common during the rainy season.

325 Distribution of WBD according to age classification is important as a result of most of health
326 related events varies with age. We found that 25-above age groups (especially old people)
327 are more susceptible to waterborne diseases, probably, due to weakened immune systems
328 not competent enough to fight against many infectious agents. The group has inadequate

329 awareness and educational background in reference to diseases in relation to water
330 contamination from direct or indirect sources. Crump *et al* (2005) support the argument,
331 ignorance on waterborne diseases may additionally play an important role in health
332 awareness in a household. Children of 0-5 age group are more vulnerable to some
333 waterborne disease due to weak immune system. Richard (2005) reported a number of
334 factors that vary with age behind association with health events such as susceptibility,
335 opportunity for exposure, latency of diseases and physiological response which affect the
336 development of diseases.

337

338 Gender of the patient is one of the most critical parameters in epidemiological studies and
339 analysis of diseases distribution. We analyzed diseases distribution according to gender
340 specification in which men are at greatest risk of experiencing WBD in Bodinga than women
341 as shown in table (4). Based on our finding, men are prone in almost all diseases with the
342 exception of cholera and skin infection in which women account high cases. This in
343 disagreement with Abdulkadir and Anandapandian, (2016) women are more prone to WBD
344 due to their role in water collection, clothes washing and other domestic activities. Men are
345 spending more time in farms which make them at a great risk of acquiring infection as they
346 are exploitation surface water for farming activities. Inherent characteristic of people,
347 acquired characteristics, activities and conditions in which they lives determine to a large
348 degree who is at risk of becoming more prone to or infected with a particular diseases
349 organisms (Richard, 2005).

350 The most probable number techniques for estimation of bacteria in water showed that there
351 is greater concentration of *Escherichia coli* in cfu/ml compared to the standard of WHO and
352 National council on water resources (NCWR) (NIS, 2007). This means water from these
353 sources are unfit for consumption and therefore, the residence of those areas are at risk of
354 being infected with water pathogens. The high occurrence of waterborne diseases in the
355 town is linked to water contamination that might be attributed due to proximity of households

356 to water sources. During the course of study it has been observed that most of the
357 communities in Bodinga town use pit latrines. The contaminants in the latrine could also be
358 leak and contaminate the water sources. The luxuriant grasses within the premises of water
359 sources attract their domestic animals to visit the area for grazing that successively leaves
360 excretes might even be the seemingly main contaminant for the drinking water available in
361 the study area. This is more likely to be rampant in the rural areas since they do not have
362 access to central waste disposal systems and effective monitoring is lacking since the study
363 area is remote.

364 **Conclusion**

365 The finding of the current studies suggests a relationship between waterborne diseases and
366 poor water quality that contributed to the unfold of diseases in addition as potential causes of
367 microbial pollution to evaluate rural drinking water supply projects in Bodinga town that is
368 presumably due to poor sanitation and hygienic conditions that embody contamination of
369 sources and open defecation within the space.

370 The study discovered some common waterborne diseases that the communities within the
371 study area are suffering, some of these diseases are contagious causing drastic impact to
372 the human within a short time interval after being contacted with the agent of the disease.
373 Children and male are observed to be more prone to the diseases than their counterpart
374 female and other age categories. A number factors contributed to spreading of diseases in
375 the area had been mentioned which includes presences of animals, the proximity of
376 households to groundwater sources and agricultural activities which in turn contributed to
377 contamination of waters sources. The data of water quality seemingly suggested the
378 concentration of *Escherichia coli* in cfu/100ml which is above the average recommended by
379 both national and international standard for thermotolerant fecal coliform bacteria. The
380 maximum fecal contamination was found in shallow well waters and the lowest concentration
381 in boreholes.

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384 **Competing of interest**

385 Authors declared that there is no competing of interest exist.

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523 Supporting information

524 **Supporting information**

525 **Bacteriological analysis S1**

526 **Number of pages 4**

527 **Number of table 3 (S3-4).**

528

529 **S1 Bacteriological analysis of water**

530 **1 Multiple Tube Test**

531 **The test comprised of three different steps**

532 **Step 1: Presumptive Test**

533 Nine tubes were set up and Label each tube with the amount of water that is to be dispensed
 534 into it i.e. (10ml in the first three test tubes, second three test tubes 1.0ml, and 0.1ml in
 535 remaining 3 test tubes). Shake water sample very well to obtain a homogeneous solution.
 536 Using pipette, transfer 10ml of sample to each first three tubes, 1.0ml of water to each of the
 537 middle set of a test tube, and 0.1ml to each of the last three tubes. Incubate all the tubes at
 538 35°C for 24 hours. Examine the tubes and record the number of tubes in each set that has
 539 gas present. Determine the MPN by referring to MPN Determination Table.

540 **Step 2: Confirmed test**

541 The positive for gas then it's probable that the sample contains coliforms and therefore the
 542 confirmed take a look at is completed by vaccinating EMB from a gas positive tube.
 543 Inoculate associate EMB plate along with your original sample of water. Incubate at 35°C for
 544 24 hours. Observe plate for coliforms (appear purple colonies with dark centers), *E. coli*
 545 appear green sheen.

546 Step

547 **Step 3: Completed test**

548 Coliform colonies from EMB were inoculated again into Lactose Broth with a Durham tube
549 and checked for gas, and inoculated on NA and checked through Gram stain for Gram-
550 negative reaction. If these tests are positive it shows that coliforms (not another gas
551 producer) are present and indicates that the water sample was contaminated.

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570 **Table S2: Monthly distribution of waterborne diseases**

571 The result of waterborne distributions in Bodinga in relation to the months and presenting
572 total number of cases account to each particular diseases identified in the area. These were
573 counted from the outpatient register in the health and record department of hospitals in
574 Bodinga town.

	Waterborne diseases						Total
	Typhoid	Dysentery	Diarrhoeae	Cholera	Gastroenteritis	Skin infection	
January	20	35	4	0	11	0	70
February	17	30	7	1	28	3	86
March	18	31	2	1	23	0	75
April	18	20	6	3	27	0	74
May	32	33	4	1	10	0	80
June	74	78	19	6	7	5	189
July	31	38	4	20	13	7	113
August	60	86	17	1	44	6	214
September	34	59	7	0	25	2	127
October	19	43	16	1	7	2	88
November	29	33	7	2	2	4	77
December	23	31	12	0	5	7	78
Total	375	517	105	36	202	36	1271

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576 **Table S3: Different mean and SE, Frequencies and percentages of some selected**
577 **waterborne diseases**

578 The values of frequency, percentages and mean average and standard error difference of
579 waterborne diseases.

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Waterborne diseases	Frequency	Percent	Mean ± Std. Error ^a
Typhoid	375	29.5	6.86± .151
Dysentery	517	40.7	6.87± .136

Diarrhoea	105	8.3	7.52± .304
Cholera	36	2.8	6.61± .302
Gastroenteritis	202	15.9	5.72± .210
Skin infection	36	2.8	8.31± .479
Total	1271	100.0	6.77± .085

585 **a- The average mean value and standard error of waterborne diseases**

586 **Table S4. The average value of faecal contamination of drinking water**
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588

Villages	Source Name	n	Ave	Stdv	Permissible limit WHO ^a /NCWR ^b /100ml
Bodinga	Borehole	10	210.4	322.14	0
	S. well ^c	10	73.2	61.73	0
Danchadi	Borehole	10	160.7	158.08	0
	S. well	10	35.1	39.90	0
Takatuku	Borehole	10	142.5	127.60	0
	S. well	10	53.9	44.37	0

589 S. well = Shallow well, Ave. = average, Stdv = standard deviation; WHO = World health
590 organization, NCWR = National council on water resources.

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