International Journal of Biochemistry and Biophysics 12(2): 11-19, 2024 http://www.hrpub.org
DOI: 10.13189/ijbb.2024.120201

Seasonal Variations in the Bacteriological Parameters of Boreholes Water in Emene, Enugu State, Nigeria

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Received August 28, 2023; Revised April 10, 2024; Accepted May 6, 2024

Cite This Paper in the Following Citation Styles

(a): *[1] Nzekwe Chidimma MaryAnn, Onuorah Samuel Chinedu , "Seasonal Variations in the Bacteriological Parameters of Boreholes Water in Emene, Enugu State, Nigeria, "International Journal of Biochemistry and Biophysics, Vol. 12, No. 2, pp. 11 - 19, 2024. DOI: 10.13189/ijbb.2024.120201.*

(b): *Nzekwe Chidimma MaryAnn, Onuorah Samuel Chinedu (2024). Seasonal Variations in the Bacteriological Parameters of Boreholes Water in Emene, Enugu State, Nigeria. International Journal of Biochemistry and Biophysics, 12(2), 11 - 19. DOI: 10.13189/ijbb.2024.120201.*

Abstract Boreholes water in Emene, Enugu State, were analyzed seasonally to determine the variations in their bacteriological quality. The total bacteria and coliforms were isolated using membrane filters. The total bacteria and coliforms, S*higella, Klebsiella, Enterobacter* and *Enterococci populations varied* during both seasons. Total bacterial loads of the water in the dry season were between 12MPN and 272MPN/100mL, total coliforms, 0MPN to 120MPN/100mL, faecal coliforms, 0MPN to 28MPN/100mL, *Enterococcus spp,* 0MPN to 19MPN/100mL, *E. coli*, 0cfu to 12cfu/mL, *Klebsiella spp,* 0cfu to 18cfu/mL, *Enterobacter* spp, 0cfu to 41cfu/mL and *Shigella spp,* 0cfu to 53cfu/mL. The total bacterial populations were between 34cfu to 272cfu/mL, total coliforms, 0MPN to 142MPN/100mL, faecal coliforms, 0MPN to 38MPN/100mL, *Enterococcus spp,* 0MPN to 12MPN/100mL, *E.coli,* 0cfu to 15cfu/mL, *Klebsiella* spp, 0cfu to 20cfu/mL, *Enterobacter* spp, 0cfu to 32cfu/mL and *Shigella* spp, 0cfu to 28cfu/mL. *Shigella* spp were predominant while Enterococcus spp had the least frequency of occurrence during both seasons. Antibiotic sensitivity test showed that chloramphenicol would be efficacious in the elimination of illnesses associated with the bacteria isolated from the selected boreholes since all the organisms were sensitive to it. The boreholes water studied should therefore be adequately treated to render them potable.

Keywords Faecal Coliform, Boreholes, Season, Membrane Filtration, Total Coliform

1. Introduction

Water is critical to the metabolism of living organisms [1]. It is the most essential commodity of humans and occupies about 97% of the earth's crust. Marine environment has 70% of water, polar ice and glacier, 21%, ground water, 0.3-0.8% while 0.009 percent is contained in rivers [2]. The availability of water is indispensable in the maintenance of ecological balance.

Water that is potable should be colourless, odourless and tasteless [3]. Its boiling and freezing temperatures are 100^{o}C and 0^{o}C respectively. It is usually obtained from underground aquifers through wells or boreholes. Ground water includes water from wells and boreholes. The water is assumed to be unpolluted because of the depth. However, boreholes water is increasingly being polluted by materials from both point and non-point sources.

Ground water including boreholes water can be contaminated by seepages from pesticides and fertilizer-applied soil, human and animal excreta, pit latrines, septic tanks, domestic, industrial and municipal wastes and refuse dumps. Eyankware *et al.* [4] observed most surface water resources accessible to households in urban areas are prone to contamination by chemical and biological contaminants which may originate from storage tanks and storm water runoff [4].

Bacteria, fungi, protozoans, viruses and parasitic worms are associated with water- borne diseases [2]. Polluted

water, poor hygiene and sanitation are some of the sources of diseases with significant mortality among individuals in low and medium income countries including Nigeria [5,6]. These biological contaminants in drinking water are responsible for various diseases including poliomyelitis, infectious hepatitis, bacillary dysentery, bilharziasis, cholera, and onchocerciasis [2].

The number of water borne disease outbreaks that have been reported in Nigeria is a clear indication that transmission of infectious agents by drinking water is a significant cause of illness [7]. Some of the symptoms of diseases transmitted by water are nausea, diarrhea, fever, vomiting, paralysis and abdominal cramps.

As a result of the paucity of public water supply systems, residents source their drinking water from boreholes. Some of these boreholes are sited in areas of poor sanitation. This work therefore studies the seasonal variations in the bacteriological parameters of selected water boreholes in Emene, Nigeria. The results will educate the inhabitants on the quality of the water they consume in the area.

2. Materials and Methods

2.1. Study Area

The work was carried out in Emene, Enugu State. Emene is located in the tropical rainforest belt of West Africa and the major occupation of the inhabitants is agriculture. It also has a rainforest climate. The relative humidity is about 75%, reaching 80% during wet season with an annual rainfall of 2000mm. The mean temperature ranges from 22℃ to 30℃ and 28℃ to 32℃ in the wet and dry seasons respectively. The study area is blessed with significant ground water resources which the dwellers rely on for their domestic, drinking and agricultural purposes. The water samples were collected from Agbani street, Nkanu street, Uga street, Ukulu Lane, Ifemeremma street, Owa street, Okolo street, Umunze street, Umuowa street, Udemaduka street, Akwaeze street, Akpeh street, Abomini close, Chukwu Ejim street and Chris Ani close.

2.2. Study Design

The research was carried out with water samples from randomly- selected fifteen boreholes, consumed by residents of Emene, Enugu State. The boreholes were sampled from January to June 2022.

2.3. Sample Collection

Sterile glass bottles (250ml), which were rinsed with the samples before collection were used. To avoid contamination during sample collection, adequate quality control measures were taken. The water was conveyed to the Laboratory of the Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka under aseptic conditions for analysis.

2.4. Samples Processing

Membrane filtration method was used. The sterile filtration apparatus was used. The water samples were thoroughly shaken before filtering one hundred milliliters through the membrane filter. The membrane filter was thereafter removed with sterile forceps and transferred aseptically to the surface of an appropriate culture medium in a Petri dish.

2.5. Total Bacterial Count

This test was performed according to the method described by Cheesbrough [8]. Nutrient agar was prepared, autoclaved, dispensed into Petri dishes and cooled. The membrane filter paper was transferred to the surface of the nutrient agar. The experiment was carried out in duplicates. The Petri dishes were incubated at 35℃ for 24hours after which the bacterial colonies were counted.

2.6. Coliform Test

2.6.1. Presumptive Coliform Test

The Most Probable Number (MPN) technique of Cheesbrough [8] was employed. Varying volumes of water were introduced into test tubes containing MacConkey broth, bromocresol purple indicator and inverted Durbam tubes and incubated at 37ºC for 48 hours. Acid and gas production indicated a positive result. The MPN of total coliforms was calculated with reference to McCrady's Probability Table.

2.6.2. Confirmed *E. Coli* Test

This was done following the method of Cheesbrough [8]. One milliliter of culture from the positive presumptive test was inoculated into tubes containing MacConkey broth, bromocresol purple and Durham tubes. Incubation was at 44.5ºC for 48 hours. Gas production indicated a positive result. The Most Probable Number of *E. coli* was obtained using McCrady's Probability Table. Aliquots from the positive presumptive test were also spread uniformly on plates of Eosin methylene blue agar. Incubation was at 35ºC for 24 hours. The detection of organisms with a greenish metallic sheen was a positive reaction.

2.6.3. Completed Coliform Test

Lactose broth and nutrient agar slants were inoculated with colonies with a greenish metallic sheen and incubated at 35ºC for 24 hours according to the scheme of Cheesbrough [8]. Gas production in the Lactose broth and the presence of Gram negative, rod shaped bacteria in the agar slants were positive results.

2.7. *Shigella Count*

One millilitre of the water sample was enriched in tubes

containing 10ml of Tetrathionate Brilliant Green broth. Aliquot (1mL) of the enriched sample was plated on *Salmonella Shigella* agar. Incubation was done at 37ºC for 48 hours. Colourless organisms were identified morphologically and biochemically as described by Cheesbrough [8].

2.8. Examination for *Enterococci*

The Most Probable Number method described by Cheesbrough [8] was used, with glucose azide broth as the growth medium. Various quantities of water were added to tubes containing the broth. Incubation of the culture was at 37ºC for 72 hours. Acid production showed a positive result. The Most Probable Number of *Enterococci* was determined from McCrady' Probability Table.

2.9. Bacterial Characterization and Identification

Gram staining, catalase, motility, indole, methyl red, voges proskauer, citrate utilization, sugar fermentation, oxidase and Urease tests were used as described by Cheesbrough [8] to characterize and identify the isolated bacteria.

2.10. Antibiotics Susceptibility Profiles of the Isolated Bacteria

This was determined as described by Nassar *et al.* [9]. A loopful of bacteria colonies from the pure culture plate was inoculated using a sterile inoculating loop into two millilitres of Muller Hinton broth. The bacterial suspension was matched with McFarland Standard using UV Spectrophotometer. The Muller Hinton agar was prepared based on the manufacturers' instructions and sterilized using an autoclave. It was introduced into culture and allowed to gel and labelled. A sterile cotton swab stick containing the broth was streaked on to the Muller Hinton agar plate in zig-zag style. Antibiotic discs: Amoxicillin (10µ), Amikacin (30µg), Chloramphenicol (10µg) and Ciprofloxacin (1µg) were placed on the agar culture using a sterile inoculation loop. The culture was incubated for 24 hours at 37ºC. The zones of inhibition of the isolates which indicated their susceptibility patterns for the drugs used were measured with a metre rule.

2.11. Data Analysis

Two way Analysis of Variance was used to analyze the data obtained. Duncan's multiple test range was employed in comparing the means of the parameters for significance at $P<0.05$.

3. Results

Table 1 showed the bacterial loads of the boreholes water in the dry season. The total bacterial populations were 12MPN to 222MPN/100mL, total coliforms, 0MPN to 120MPN/100mL, faecal coliforms, 0MPN to 28MPN/100mL, *Enterococcus spp*, 0MPN to 19MPN/100mL, *E.coli*, 0cfu to 12cfu/mL, K*lebsiella* spp, 0cfu to 18cfu/mL, *Enterobacter* spp, 0cfu to 41cfu/mL and *Shigella* spp, 0cfu to 53cfu/mL.

Table 2 showed the bacterial loads of the boreholes water in the wet season. The total bacterial loads were from 34cfu to 272cfu/mL, total coliforms, 0MPN to 142MPN/100mL, faecal coliforms, 0MPN to 38MPN/100mL, Shigella spp, 0cfu to 28cfu/mL, Enterococcus spp, 0MPN to 12MPN/100mL, E.coli, 0cfu 15cfu/mL, Klebsiella spp, 0cfu to 20cfu/mL and Enterobacter spp, 0cfu to 32cfu/mL.

The average bacterial loads of the boreholes water and their comparison with WHO [6] Standard in the dry and wet seasons are presented in Table 3. The average total bacterial counts were 109.86cfu to 142.66cfu/mL, Shigella spp, 20.33cfu to 10.20cfu/mL, faecal coliforms, 8.20MPN to 14.66MPN/100mL, Enterobacter spp, 0cfu to 36cfu/mL, E.coli, 3.60cfu to 7.46cfu/mL, Enterococcus spp, 9.33cfu to 14.73cfu/mL, total coliforms, 41.53MPN to 56.66 MPN/100mL and Klebsiella spp, 5.33cfu to 13.00cfu/mL.

The colony morphology and biochemical features of the bacteria from the boreholes water in the dry and wet seasons are shown in Table 4. They were Klebsiella spp, Shigella spp, Enterobacter spp, Enterococcus spp and Escherichia coli.

Table 5 showed the sensitivity patterns of the bacteria from the boreholes water in both seasons to antibiotics. All the isolates were sensitive to Chloramphenicol and resistant to Amikacin and Tetracycline.

The frequency of isolation of bacteria in the boreholes water in the dry season is presented in Figure 1. Enterococcus spp (14.30%), Enterobacter spp (29.01%), Klebsiella spp (10.29%), Shigella spp (40.50%) and Escherichia coli (5.90%) occurred in the water samples.

Figure 2 showed the frequency of isolation of bacteria in the boreholes water in the wet season. Escherichia coli (16.98%), Shigella spp (23.74%), Enterobacter spp (21.54%), Klebsiella spp (30.98%) and Enterococcus spp (6.76%) were isolated from the water samples.

Figure 3 showed the frequency of isolation of bacteria in the boreholes water in both seasons. Escherichia coli (10.98%), Enterobacter spp (25.58%), Klebsiella spp (19.80%), Shigella spp (32.80%) and Enterococcus spp (10.84%) occurred in the samples.

Table 1. Bacterial loads of the boreholes water in the dry season

S/N	Borehole location	Total Bacteria (cfu/mL)	Total Coliforms (MPN/100mL)	Faecal Coliforms (cfu/mL)	Shigella (cfu/mL)	Enterococcus (MPN/100mL)	Enterobacter (cfu/mL)	E.coli (cfu/mL)	Klebsiella (cfu/mL)
1	Agbani Street	72	0	0	37	0	0	0	0
2	Nkanu Street	20	0	0	10	0	0	0	0
3	Uga Street	222	120	4	14	15	41	4	18
4	Ukulu Lane	12	12	0	0	10	0	0	0
5	Ifemeremma	54	22	0	13	9	4	0	11
6	Owa Street	115	35	11	21	8	10	9	10
7	Okolo Street	135	64	8	0	6	41	5	6
8	Umunze Street	143	41	15	36	3	20	7	0
9	Umuowa Street	78	0	0	53	19	0	0	0
10	Udemaduka Street	194	96	20	25	0	17	12	18
11	Akwaeze Street	138	52	12	28	12	24	2	5
12	Akpeh Avenue	47	9	0	5	19	1	0	2
13	Abomini Close	203	106	21	18	0	31	8	15
14	Chukwu Ejim	37	6	4	5	0	0	3	2
15	Chris Ani Close	178	48	28	30	17	32	4	3

Table 2. Bacterial loads of the boreholes water in the wet season

S/N	Borehole location	Total Bacteria (cfu/mL)	Total Coliforms (MPN/100mL)	Faecal Coliforms (cfu/mL)	Shigella (cfu/mL)	Enterococcus (MPN/100mL)	Enterobacter (cfu/mL)	E.coli (cfu/mLl)	Klebsiella (cfu/ml)
1	Agbani Street	87	31	0	28	0	0	0	20
2	Nkanu Street	42	0	0	9	0	0	15	0
3	Uga Street	271	142	32	0	7	32	8	19
4	Ukulu Lane	34	0	11	0	0	0	5	0
5	Ifemeremma	106	53	5	0	3	1	10	15
6	Owa Street	187	54	14	18	0	5	0	22
7	Okolo Street	144	86	11	0	0	25	21	7
8	Umunze Street	207	74	18	28	7	10	3	10
9	Umuowa Street	82	16	0	0	0	0	0	8
10	Udemaduka Street	272	103	23	20	4	8	21	22
11	Akwaeze Street	146	64	17	22	12	18	3	13
12	Akpeh Avenue	50	19	0	0	0	0	0	7
13	Abomini Close	264	134	37	12	0	18	15	30
14	Chukwu Ejim Street	61	15	13	0	0	0	9	12
15	Chris Ani Close	187	59	38	16	11	23	8	10

Table 3. Comparison of the average bacterial counts of the boreholes water in the dry and wet seasons with WHO [6] Standard

Season	Total Bacteria(cfu/mL)	Total Coliforms (MPN/100ML)	Faecal Coliforms (cfu/mL)	Shigella (cfu/mL)	Enterococcus (MPN/100mL)	Enterobacter (cfu/mL)	E.coli (cfu/mL)	Klebsiella (cfu/ml)
Dry season	109.86±17.99	41.53±10.71	8.20±2.40	20.33±.60	7.20±1.94	14.73±4.06	3.60±1.00	5.33±1.72
Wet season	142.66±21.96	56.66±11.65	14.60±3.40	10.20±1.11	2.93±1.11	9.33±2.90	7.46±1.63	13.00±2.17
Mean difference	-32.8	-15.133	-6.4	10.13	4.266	5.40	-3.866	-7.666
P-value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WHO Standard	0	0	0	0	0	0	0	0

Table 4. Colony morphology and biochemical features of the bacteria from the borehole water in the dry and wet seasons

Isolate	Form	Surface	Colour	Margin	Elevation	Opacity	Gram	Cat	Mot	Ind	MR	VP	Cit	Lac	Glu	Suc	Fru	Mal	Ox	Ur	Identity
A	Irregular	Glistening	Cream	Entire	Raised	Opaque	- Rod	+	-	-	+	-	+	+	+	+	-	+	-	+	*Klebsiella spp*
B	Circular	Smooth	Greyish	Entire	Convex	Translucent	- Rod	+	-	Var	+	-	-	-	+	-	+	Var	-	-	*Shigella spp*
C	Circular	Shiny	White	Entire	Convex	Moist	- Rod	+	+	-	-	+	+	+	+	+	+	+	-	-	*Enterobacter spp*
D	Circular	Smooth	Cream	Entire	convex	Opaque	+Coccus	-	-	-	-	+	-	+	+	+	-	+	-	-	*Enterococcus spp*
E	Circular	Smooth	Whitish	Entire	Convex	Translucent	- Rod	+	+	+	+	-	-	+	+	var	-	-	-	-	*Escherichia coli*

Gram= Gram reaction, Cat= Catalase, Mot= Motility, Indole= Indole, MR= Methyl red, VP= Voges Proskauer, Cit= Citrate, Lac= Lactose, Glu= Glucose, Suc= Sucrose, Fru= Fructose, Mal= Maltose, Ox= Oxidase, Ur= Urease, Var= Variable

Table 5. Sensitivity patterns of the bacteria (zone of inhibition in mm) to antibiotics

Antibiotics Names	Disc content (µg)	Standard values			*E. coli*	*Enterococcus spp*	*Klebsiella spp*	*Enterobacter spp*	*Shigella spp*
		R≤	I	S≥					
Amoxicillin	10	14	-	15	R(5.8)	R(6.0)	S(16.3)	R(3.9)	R(5.0)
Amikacin	30	15	16-18	19	R(6.5)	R(5.7)	R(8.1)	R(7.2)	R(6.2)
Chloramphenicol	10	14	-	15	S(16.1)	S(15.0)	S(15.1)	S(17.0)	S(17)
Ciprofloxacin	1	16	17-19	20	I(17.3)	R(4.4)	R(4.1)	R(7.1)	R(16.6)
Tetracycline	10	19	-	20	R(9.4)	R(8.8)	R(6)	R(6.1)	R(7.3)

Key: R = Resistance, S= Sensitive, I = intermediate

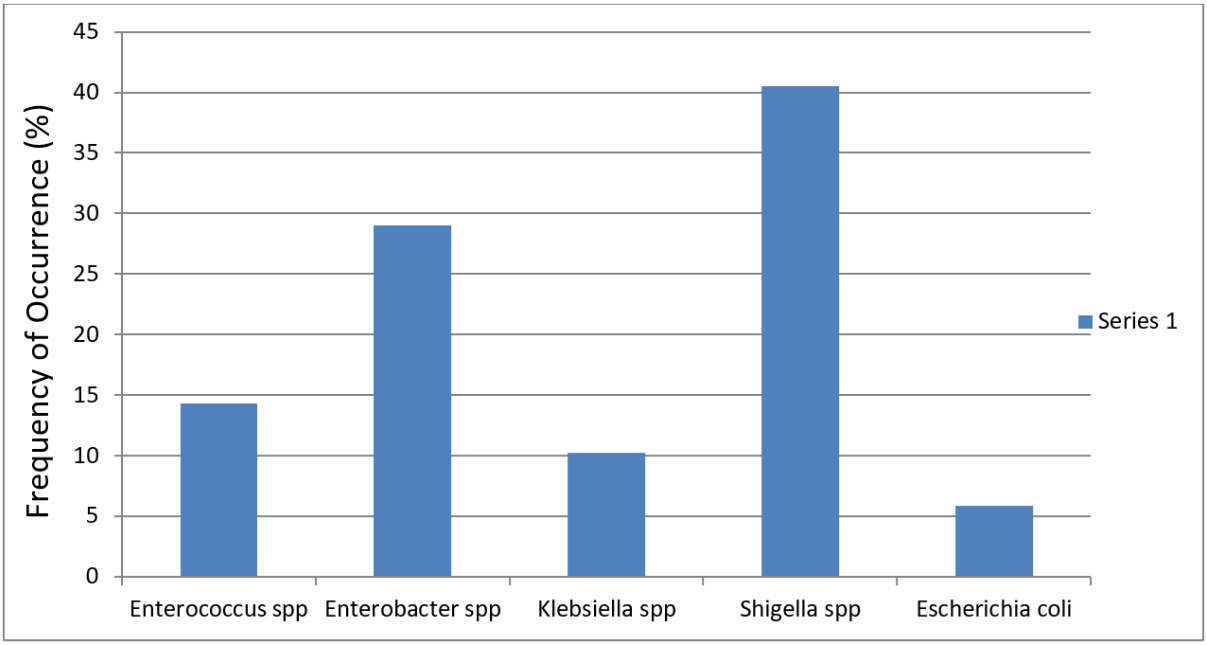

Figure 1. Frequency of isolation of bacteria in the boreholes water in the dry season

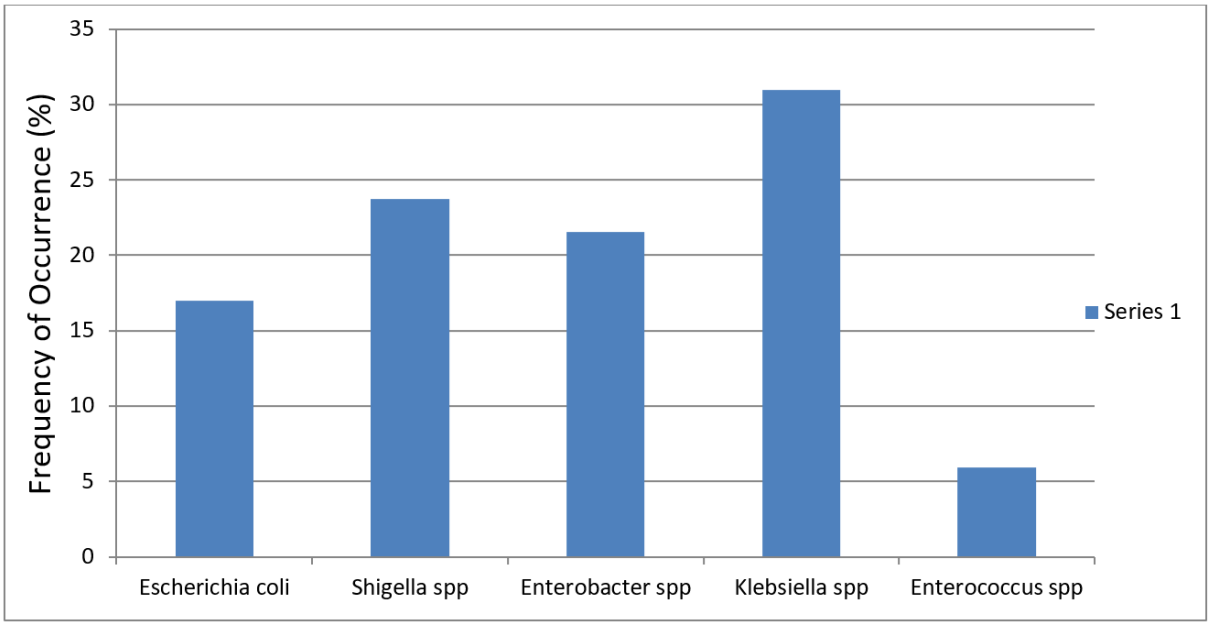

Figure 2. Frequency of isolation of bacteria in the boreholes water in the wet season

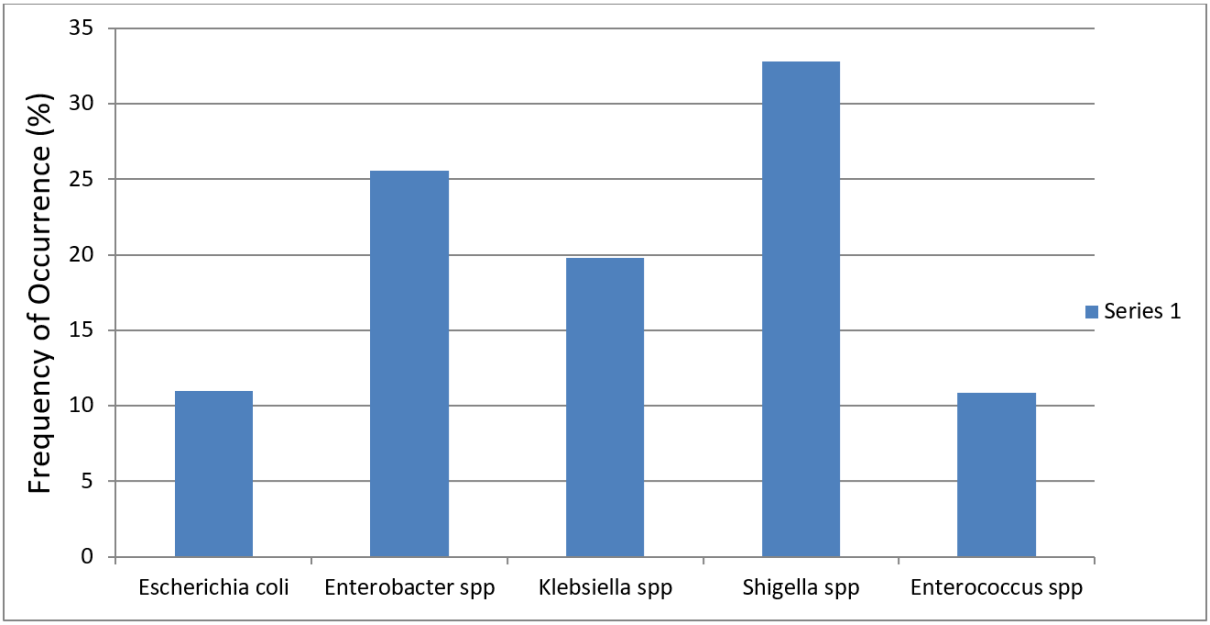

Figure 3. Frequency of isolation of the bacteria in the boreholes water in both seasons

4. Discussions

The water sampled in the dry season (Table 1) was contaminated with bacteria. The organisms were in excess of the number allowed by regulatory agencies. This result is similar to that of Ukpong and Peter [10] on the physicochemical and bacteriological analyses of drinking. The bacterial growth could be a result of the unavailability of the required nutrients as well as the unconducive environment needed for bacterial growth.

The bacterial counts of the boreholes water in the wet season were higher than those of the dry season. They were also higher than the stimulated level (Table 2). These results agreed with those of Krishna *et al*. [11] concerning the physicochemical and bacteriological parameters of Kavari River in India. This indicated that the boreholes were heavily contaminated in the wet season as a result of flow of undesirable substances in to the body of water.

The statistical analysis showed that the total bacterial counts, total coliforms, faecal coliforms, *Shigella* spp, *Enterococcus* spp, *E.coli*, *Enterobacter* spp and *Klebsiella* spp in both dry and wet season samples increased significantly (p value= 0.00), indicating that there were seasonal variations in their counts during the dry and wet seasons (Table 3). Coliforms are indicators of the sanitary quality of water. They are the bacteria frequently recovered from water and are linked to diseases such as diarrhea, typhoid and dysentery. Coliforms have also been associated with mortality across the whole world especially in Africa [12].

The total bacterial count should not exceed 100cfu/mL in accordance with WHO Standard [5]. The average bacterial counts of all the sampled boreholes water failed to conform to the WHO Standard for drinking water (Table 3). Total coliforms, faecal coliforms, *Shigella* spp, *Klebsiella* spp, *Enterobacter* species, *E.coli* and *Enterococcus* species occurred in the samples. This indicated that the boreholes water examined in Emene, Enugu State was not safe for drinking (Table 3). This result is in agreement with the findings of Shittu *et al*. [13] on the physicochemical and bacteriological analyses of water used for drinking and swimming purposes and Engwa et al. [14] that analyzed potable water sources physicochemically and microbiologically.

The detection of faecal coliforms in this study showed the water samples were contaminated with faeces of either human or animal origin. The result also agreed with the observations of Akubuenyi *et al*. [15] who assessed major sources of water for domestic uses microbiologically and physicochemically as well as the report of the comparative analysis of three borehole water sources conducted by Okoro et al. [16]. The faecal coliforms are pathogenic bacteria and can cause several infectious diseases in humans such as cholera, dysentery, gastroenteritis and urinary tract infections. The presence of water is a public health hazard since they are implicated in many water-borne diseases.

Klebsiella spp, *Enterobacter* spp and *Enterococcus* spp isolated from the boreholes water are inhabitants of soil and vegetation and are therefore not of faecal origin. They are referred to as atypical coliforms. The isolation of *E. coli* is the most reliable indication of the pollution of water by faeces and also indicates the presence of other pathogenic organisms in the gastrointestinal tract as reported by Akubuenyi *et al*. [15]. The bacteria in the boreholes water in both seasons were characterized and identified as E.coli, Shigella spp, Klebsiella spp, Enterococcus spp and Enterbacter spp (Table 4). Obeta and Mamah [17] also isolated similar bacteria in their study of the influence of environmental factors on the physicochemical and

bacteriological quality of well and borehole water in rural communities.

Enterococcus spp (14.30%), *Enterobacter* spp (29.01%), *Klebsiella* spp (10.29%), *Shigella spp* (40.50%) and *Escherichia coli* (5.90%) were isolated from the boreholes water in the dry season (Figure 1).Shigella spp had the highest frequency of isolation. The frequency of isolation of the bacteria in the boreholes water in the wet season (Figure 2) is thus: *Escherichia coli* (16.98%), *Shigella* spp (23.74%), *Enterobaccter* spp (21.54%), *Klebsiella* spp (30.98%) and *Enterococcus* spp (6.76%) were recovered from the water in the wet season. The result indicated that the non-faecal coliform bacteria, Klebsiella spp were dominant.

Escherichia coli (10.98%), *Enterobacter* spp (25.58%), *Klebsiella* spp (19.80%), *Shigella* spp (32.80%) and *Enterococcus* spp (10.84%) occurred in the water in both seasons (Figure 3). The study showed that Shigella spp were the most frequently recovered bacteria from the boreholes water.

All the bacteria detected in the boreholes water were susceptible to Chloramphenicol out of the antibiotics used for the study (Table 5). This indicated that chloramphenicol can be effective in the cure of infections caused by the bacteria isolated from the boreholes water studied in Emene, Enugu State.

5. Conclusions

The analyses showed that most of the samples analysed during both seasons were contaminated with bacteria which have been implicated in water-borne diseases. The presence of total coliforms and faecal coliforms is indicative of poor sanitation within the surroundings of the boreholes. The isolation of coliform bacteria is a confirmation of the presence of pathogens in the boreholes water, hence the need for adequate treatment before consumption. Water samples from the affected boreholes should also undergo regular bacteriological analyses.

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