

ANTIBIOTIC SUSCEPTIBILITY OF ENTEROBACTERIACEAE ISOLATED FROM DOMESTIC WATER TANKS IN BIDA, NIGER STATE, NIGERIA

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ABSTRACT

Most communities (rural and sub urban) in Nigeria get their water supply from untreated sources like wells, streams, rivers and ponds that are mostly contaminated with pathogenic bacteria. This exposes people to gastroenteritis such as Typhoid fever, dysentery, diarrhea and cholera. Antibiotic Susceptibility of Enterobacteriaceae isolated from domestic water tanks in Bida town to some antibiotics was investigated. A total of forty (40) water samples were collected from different domestic water tanks located in the four (4) cardinal points of Bida town and subjected to physicochemical and bacteriological analysis. The bacterial isolates identified were further subjected to antibiotic susceptibility test using Septrin, Streptomycin, Chloramphenicol, Sparfloxacin, Ciprofloxacin, Amoxicillin, Augmentin, Gentamycin, Perfloxacin and Tarivid. The temperature and pH of the water samples favored the growth of Enterobacteriaceae. Conductivity, Biochemical oxygen demand (BOD), Chemical oxygen demand (COD), Total suspended solid, Total dissolved solid, Calcium, Zinc, Magnesium, and Nitrate of water samples were all above the WHO acceptable limit for drinking water. The result revealed that the total viable count of bacteria ranged from 1.0×10^4 cfu/ml to 9.9×10^4 cfu/ml in Eyagi and Banyagi (west) and GRA (South) respectively. Bacteria species isolated from the water samples included *Escherichia coli*, *Salmonella sp*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, and *Proteus*. The most frequently isolated bacteria were *Escherichia coli* and *Salmonella sp*. with percentage frequency of 27.77% while the least isolated bacterium was *proteus sp* (4.16%). The percentage susceptibility pattern of the bacteria was: *E. coli* (30%), *Salmonella sp*. (20%), *Enterobacter sp*. (10%), *Klebsiella sp*. (20%), *Pseudomonas sp*. (20%), while *Proteus sp*, was 100% resistant to all antibiotics. The result of this findings is an indication that water stored in domestic tanks in Bida town is contaminated with different species of Enterobacteriaceae hence not safe for human consumption.

Key words: Domestic water Tank, Pathogens, Enterobacteriaceae, Viable count, Antibiotic Susceptibility, Bida

INTRODUCTION

Water is one of the most essential needs for the existence of all living organisms on earth; this is because day to day activities of living things require water in different forms. Water is effectively and efficiently

put into use by man, plants, animals, and microorganisms (Sofola and Lawal, 1983 :). In the microbial world, no single microorganism has been discovered to be active at extreme lack of water. For the

singular fact that man cannot survive without water, it is of paramount importance to monitor domestic water supply (Prescott *et al.*, 1999).

In Nigeria, especially in the rural and sub – urban communities, water for drinking and other domestic uses is mostly obtained from wells, streams, ponds and rivers (Oyetao *et al.*, 2007). Reliance on these untreated sources of water is due to lack of potable water (pipe born water). The wells and streams are subject to contamination with pathogenic bacteria because of their proximity to human activities. Poor construction and inadequate protection of wells, closeness of water body to refuse dumps sites, and latrines, bathing and washing inside the streams and rivers and disposal of industrial and domestic wastes into water predispose them to contamination (Shuyal, 2003).

Non availability of potable water in most Nigerian communities has become a critical and urgent problem and it is a matter of great concern to the people (Okonkwo *et al.*, 2008). Increase in human population exerts an enormous pressure on the provision of safe drinking water. Unsafe water is a public health threat that place people at risk for a host of gastro enteritis diseases like diarrhea, bacillary dysentery, Typhoid fever and cholera as well as chemical intoxication (Hughes and Koplun, 2005). These infections are common in places where there is lack of hygienic portable water supply and improper sewage disposal system (Ayotebi, 2000; Ben Aissa., 2007). Consumption of non-potable water has lethal effect on children particularly those less than five years of age. World health organization, 2005

revealed that diarrhea accounted for about 17% of death in children (0 – 5 years) from 2000 to 2003 (Ben Aissa *et al.*, 2007; Olayemi *et al.*, 2005). Shortage of water however have led people to store water in tanks for a very long period of time. However, bacteria species that initially contaminate the water tend to multiply during storage, hence making stored water unsafe for consumption (Edema *et al.*, 2001). The present study aims to investigate susceptibility of enterobacteriaceae isolated from domestic water tanks in Bida, Niger State to some common antibiotics.

MATERIALS AND METHOD

Study area

The study was conducted in Bida town, the headquarters of Bida local government area, Niger State. Bida is the second largest town in Niger state with a land area of approximately 51km and an estimated population of 289,985,000 (NPC, 2010). Bida is a dry, arid town located close to Gbako River and lies at the intersection of roads from Jebba, Zungeru and Agaie between Latitude 9011E to 9081E, and Longitude 5071N to 6017N.

Collection of samples

A total of forty (40) water samples were collected from the study area. Five areas were chosen from each of the four cardinal points of the town, and two samples were collected from two tanks in each area and bulked together as a sample. The samples were labeled accordingly and transported to Microbiology Laboratory, IBB University Lapai. The samples were processed in accordance with the guidelines for laboratory analysis of Enterobacteriaceae provided by Cheesbrough, 2006.

Physicochemical Analysis

The physical and chemical properties of the water samples were analyzed using standard methods. The physicochemical parameters analyzed include pH using pH meter, temperature was measured using glass thermometer. Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were determined using respirometric method and reflux titrimetric method respectively, Total Dissolve Solids (TDS) was measured by evaporation method, while Total Suspended Solids (TSS) was measured by gravimetric method.

Total viable count

Water samples were subjected to a tenfold serial dilution. Dilutions 10^4 and 10^5 were chosen and inoculated onto nutrient agar plates and incubated at 37°C for 24 hours. Colonies that appeared on the plates were counted and expressed as cfu/ml.

Most probable number (MPN)

Most Probable Number test was carried out to estimate the population density of viable bacteria in the test water samples. The three steps of MPN (presumptive, confirmatory and completed tests) were carried out and the tubes with positive result after incubation period of presumptive test was selected for confirmation test by pour plating on Erlenmeyer's Broth (EMB) agar and incubated at 35°C for 24-48 hours. Isolates obtained from the pour plate were sub-cultured into Nutrient Agar (NA) plates to obtain a pure culture. Isolates thus obtained on the nutrient agar plates, were transferred onto nutrient agar slants then subjected to gram stain reaction and various biochemical tests such as nitrate utilization,

indole, oxidase, catalase, coagulase, methyl red and sugar fermentation tests. Characteristics of the isolates were compared with those of known taxa.

Antibiotic susceptibility test

Antibiotics susceptibility test was carried out on each isolate using multiple sensitivity disc. The isolates were diluted to 0.5 McFarland's standard and streaked on the surface of solidified nutrient agar using sterile swab stick. Antibiotic discs were placed on the center of inoculated plates and incubated at 37°C for 24 hours. Zones of inhibition were measured to the nearest millimeter (mm) using metric transparent ruler (Cheesbrough, 2000). The percentage susceptibility was then determined by dividing the number of isolates susceptible to a particular antibiotic by the total number of isolates considered and then multiplied by 100.

Results

Table 1 shows the result of the physicochemical properties of water samples. Water temperature ranged from 11.2°C in Lalemi to 26.5°C in Dokodza and Yissanta. Low-cost and Wadata had the lowest turbidity of 0.00 NTU while the highest turbidity (0.09 NTU) was recorded in Efugeaye and Dokodza. The pH ranged from 6.4 in Yissanta to 7.5 in Wadata and area 2. The result also showed an electrical conductivity range of 0.06 dsm^{-1} in Banyagi (west) to 0.14 dsm^{-1} in Area2 (north). Highest biochemical oxygen demand (185 mg/l) was recorded in Laruta while Eyagi had the lowest BOD (120 mg/l), Bangaye in the western part of Bida and Tadafu in the east. Lowcost and Yissanta recorded the lowest chemical oxygen demand (COD) (150 mg/l) while the highest

value was recorded in Wadata (125mg/l). Banwuya in the south of Bida had the highest value of total dissolved solids (0.8mg/l), Area 2, lowcost, lafiya and Dokodza had 2.0mg/l, and the lowest (1.0mg/l) was recorded in new market and Yissanta.

Total viable count of 1.0×10^4 cfu/ml in Eyagi and Banyagi in the western part of the town while the highest value (9.9×10^4) cfu/ml) was recorded in GRA located in south of Bida. The lowest MPN (11/100) was recorded in Eyagi and Banyagi while the highest value (93/100) was recorded in GRA and Banwuya (table 2).

Enterobacteriaceae isolated from the water samples were *Escherichia coli*, *Salmonella sp.*, *Enterobacter sp.*, *Klebsiella sp.*, *Pseudomonas sp.*, *Proteus sp.* *E. coli* and *Salmonella sp.* have the highest frequency of occurrence of 20 each, *Enterobacter*, *Klebsiella*, *Pseudomonas* and *proteus* species had occurrence values of 10, 9, 8, and 5 respectively. The most frequently isolated bacteria were *E. coli* and *Salmonella* with percentage frequency of 27.77% each while the least isolated bacteria were *proteus sp.* with 6.95% frequency (Table 3).

Table 1: Physicochemical properties of the water samples

Site	Temp	pH	TBT	CD	BOD	COD	TSS	TRPC	COLOR	TDS	Ca	Fe	Mg	Zn	No3
Laruta	17.1	5.0	0.02	0.08	185	114	0.0	C	CL	0.0	0.24	0.112	2.11	0.00	0.00
Edogifu	19.0	5.8	0.03	0.10	126	120	0.0	C	CL	0.0	0.23	0.11	2.08	0.11	0.11
Eyagi	16	5.6	0.01	0.08	120	121	0.5	C	CL	1.0	0.21	0.113	2.00	0.13	0.13
Barachita	20	5.0	0.01	0.10	124	121	0.3	C	CL	1.0	0.22	0.112	2.00	0.12	0.12
Bangaye	15.5	5.3	0.03	0.06	120	123	0.5	C	CL	0.0	0.23	0.111	1.00	0.00	0.11
Lalemi	11.2	5.2	0.01	0.10	128	122	0.9	C	CL	0.0	0.21	0.116	2.16	0.06	0.00
Esso	13.1	5.7	0.01	0.09	125	118	0.12	C	CL	1.0	0.16	0.114	2.13	0.02	0.06
Iyaruwa	18	4.8	0.06	0.12	125	111	0.2	C	CL	2.0	0.18	0.113	2.11	0.12	0.02
Tadafu	15.9	4.5	0.07	0.10	120	120	0.2	C	CL	0.0	0.23	0.113	2.13	0.12	0.12
Ndazabo	19	5.1	0.01	0.09	121	116	1.0	C	CL	0.0	0.22	0.112	2.14	0.18	0.12
Banwuya	20.1	6.0	0.00	0.09	132	105	0.03	C	CL	2.0	0.21	0.110	0.32	0.14	0.18
GRA	19.5	6.4	0.00	0.09	130	125	0.02	C	CL	0.0	0.21	0.113	2.16	0.13	0.14
Efugiaye	19.3	5.3	0.09	0.13	128	115	0.2	C	CL	1.0	0.18	0.016	2.18	0.14	0.13
Dokodza	19.8	5.0	0.06	0.09	130	120	0.0	C	CL	2.0	0.16	0.111	1.23	0.13	0.14
Yissanta	16.3	6.4	0.07	0.14	128	121	0.0	C	CL	0.8	0.20	0.116	1.32	0.11	0.13
Lowcost	21.2	5.3	0.07	0.10	130	118	0.01	C	CL	4.0	0.18	0.111	1.32	0.16	0.11
Wadata	23	4.8	0.10	0.09	132	120	0.1	C	CL	0.0	0.23	0.116	1.36	0.11	0.16
N/market	25	5.1	0.08	0.10	128	121	0.02	C	CL	0.0	0.19	0.114	1.32	0.13	0.13
Lafiagi	26.5	5.2	0.08	0.12	130	122	0.7	C	CL	2.0	0.23	0.112	1.26	0.14	0.14
Area2	26.5	3.7	0.02	0.08	131	105	0.01	C	CL	1.0	0.23	0.116	1.48	0.13	0.13

Key: CD=Conductivity, BOD=Biochemical Oxygen Demand, COD=Chemical Oxygen Demand, TSS=Total Suspended Solid, TBT=Total Biochemical Turbidity, TRPC=Transparency, TDS=Total Dissolved Solid, Ca=Calcium, Fe=Iron, Zn=Zinc, Mg=Magnesium, C=Clear, CL=Colorless.

Table 2: Total viable count and most probable number of the water samples

Site	TVC	MPN
Laruta	2.0x10 ⁴	15
Edogifu	4.1x10 ⁴	28
Eyagi	1.8x10 ⁴	14
Darachita	1.4x10 ⁴	11
Banyagi	1.1x10 ⁴	11
Lalemi	3.7x10 ⁴	39
Esso	1.6x10 ⁴	9
Iyaruwa	5.3x10 ⁴	39
Tadafu	2.6x10 ⁴	23
Ndazabo	2.0x10 ⁴	20
Banwuya	9.0x10 ⁴	93
GRA	9.9x10 ⁴	93
Efugiaye	6.2x10 ⁴	39
Dokodza	5.0x10 ⁴	43
Yissanta	4.0x10 ⁴	39
Lowcost	7.6x10 ⁴	43
Wadata	4.8x10 ⁴	39
N/market	9.4x10 ⁴	64
Lafiayagi	5.6x10 ⁴	28
Area 2	6.0x10 ⁴	23

Table 3: Frequency of Occurrence of Bacteria Isolates from Domestic Water Tanks Sample

Bacteria	Number of isolates	Percentage occurrence (%)
<i>Escherichia coli</i>	20	27.78
<i>Salmonella</i> sp.	20	27.78
<i>Enterobacter</i> sp.	10	13.88
<i>Klebsiella</i> sp.	9	12.50
<i>Pseudomonas</i> sp	8	11.11
<i>Proteus</i> sp.	5	6.95
TOTAL	72	100

Antibiotic susceptibility pattern of bacteria isolates (%)

The bacteria isolates were tested for susceptibility to ten (10) antibiotics namely Amoxicillin, Augmentin, Ciprofloxacin, Chloramphenicol, Perfloxacin, Tarivid, Gentamycin,

Spayloxacin, Septin and Streptomycin. Antibiotic susceptibility pattern revealed that except proteus specie, all other bacteria tested in this study were susceptible to one antibiotic or the other. Table 4 illustrates percentage resistant and susceptibility

pattern of isolates to the antibiotics. Eighteen out of twenty *E. coli* isolates (90%) was susceptible to three antibiotics. Three of the 18 *E. coli* isolates were susceptible to Amoxicillin 3(15%), 5(20%) to Augmentin and 10(50%) to Ciprofloxacin. Only *Salmonella* sp. (50%) was susceptible to

Chloramphenicol, *Klebsiella*, *Pseudomonas* and *Salmonella* species were susceptible to Perfloxacin and Tarivid. *Enterobacter* sp. (10%) was susceptible to Amoxicillin alone. All isolates were resistant to Gentamycin, Spayloxicin, septrin and streptomycin.

Table 4: Antibiotic susceptibility pattern of bacteria isolates (%)

Antibiotic	Test isolate					
	<i>E.coli</i> (n=20)	<i>Enterobacter</i> (n=10)	<i>Klebsiella</i> (n=9)	<i>Proteus</i> sp (n=5)	<i>Pseudomonas</i> sp (n=8)	<i>Salmonella</i> sp (n=20)
Amoxicillin	3(15%)	1(10%)	5(55.6%)	R	4(50%)	10(50%)
Augmentin	5(20%)		R	R	R	R
Ciprofloxacin	10(50%)	R	R	R	R	R
Chloramphenicol		R	R	R	R	6(30%)
Perfloxacin	R	R	2(22.22%)	R	2(25%)	2(10%)
Tarivid	R	R	2(22.22%)	R	2(25%)	2(10%)
Gentamycin	R	R	R	R	R	R
Spayloxicin	R	R	R	R	R	R
Septtrin	R	R	R	R	R	R
Streptomycin	R	R	R	R	R	R

R=resistant, n=number isolated

Discussions

Domestic water supply in Bida town is mostly from contaminated sources; even those with plastic water tanks in their homes get their water supply from the commercial water sellers. This water is direct from the source with very high load of microorganisms and unfavorable physical and chemical parameters which The physicochemical properties of water bodies have direct effect on the rate of

have a lot of consequences on our health and the environment.

Plastic tanks cannot be treated with heat, hence result to accumulation of pathogenic microorganisms such as *Salmonella* species, *E.coli* and vibrio at the bottom of water tanks and sometimes attached to the wall of tanks as microbial biofilms (Henok *et al*, 2016).

microbial growth (Pandey, 2003). The temperature ranged of 11.2°C in (East 1) to

26.5°C in South 4 and South 5 recorded in this study favors the growth of heterotrophic bacteria species in water sample. The pH values recorded from all samples studied are below the world health organization (WHO, 2012) standard of 6.5-8.5. Except low-cost, Wadata and Area 2 that had pH within the acceptable range satisfactory for consumers. Biochemical oxygen demand (BOD) ranges from 0 to 53.2mg/L which represent the amount of oxygen the microbes need to stabilize biologically oxidizable matter. All the trace elements investigated in this study are of paramount importance to man. However, the concentration is likely to increase and get above the acceptable limit. In this study, all the physicochemical parameters analyzed are not within the acceptable range. The result of this study concurs with findings of Emre *et al.*, 2019 who recorded low pH and high values of turbidity, Ammonium, dissolved oxygen and total hardness in drinking and domestic water in Corum/Turkey. The total viable count of bacteria and MPN/100 of water in table 2 showed that even the lowest count in Eyagi and Banyagi exceeded the maximum limit of 0-5 cfu/ml. This may be as a result of inadequate maintenance of water tanks such as cleaning of tank on regular basis. Lack of well fitted lid can also cause contamination of water inside the tank; it could also be as a result of contamination of water from the so. These results agree with the result of Adeoye *et al.*, 2012 who reported high coliform count of 190 MPN per 100mL in a research conducted from Dema, yola. However, from the result above shows that the water sources from the study area were all contaminated. Therefore, inadequate safe drinking water, lack of proper sewage disposal system and drainage, inadequate proper environmental

sanitation measures will account for a reasonable number of water borne diseases that may claim millions of life every year in developing countries (Zamzaka *et al.*, 2004). Most domestic water is often contaminated where there is high concentration of physical and chemical parameter of water above or below the standard. The occurrence of these organisms in this study may pose an implication for public health. Species of the organisms may be associated with human intestinal diseases. However, this result revealed that the population of Enterobacteriaceae is above the recommendation of world health organization (WHO, 2010) of zero coliform in 100mL water sample (WHO, 2004). In vitro antibiotic susceptibility pattern shows *E. coli* to be sensitive to Ciprofloxacin, Amoxicillin and Augmentin (shows the highest percentage sensitivity pattern) but resistant to Septrin, Chloramphenicol, Sparfloxacin, Tarivid, Streptomycin, Gentamycin and Perfloracin. *Pseudomonas sp.* was more sensitive to Amoxicillin (50%) and 25% each to Gentamycin and Tarivid. Enterobacter was sensitive to Amoxicillin but, resistance to Augmentin, Streptomycin, Gentamycin, Septrin, Chloramphenicol, Sparfloxacin and Ciprofloxacin. *Salmonella sp.* shows the same sensitivity pattern with *Pseudomonas sp* and *Klebsiella* while Enterobacter had 10% sensitivity. *Proteus sp* shows total resistant to all antibiotics. This result is similar to the study of Bello *et al.*, 2013 who reported low level of antibiotic resistance among the Bacteria isolated from borehole water.

CONCLUSION

The presence of high coli form counts in water tanks can be attributed to poor hygiene and favorable temperature for bacterial

growth. This is an indication of poor sanitary integrity of the water tanks. Hence, these water samples are absolutely unfit for direct drinking without any essential treatment. Conclusively, from the result of this study, the people of Bida town may be at high risk of contracting water borne diseases such as cholera, diarrhea, typhoid fever, and skin rashes. Susceptibility pattern of the isolates to the tested antibiotics showed that amoxicillin, Augmentin, ciprofloxacin, Perfloxacin and Tarivid can be used for treatment of Enterobacteriaceae.

Recommendation

After careful observation of the findings of this study, we recommend that sources of water for domestic purposes such as boreholes, water pipes and wells be managed properly. Domestic water tanks should be handled properly and clean regularly to prevent bacterial contamination of water. Personal and environmental sanitary practices should be employed and maintained around the tanks. Boiling of water before drinking would prevent the incidence of water borne or any health-related diseases. Government should organize a program for educating the general public on where and how to sites a borehole, waste disposal, sewage treatment, so as to prevent contamination of drinkable water.

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