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## ANTIBIOTIC SUSCEPTIBILITY PATTERN OF SALMONELLA SPECIES AND OTHER MICROORGANISMS ISOLATED FROM SACHETS WATER SOLD AND CONSUMED IN NIGER DELTA REGION OF NIGERIA

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### ABSTRACT

Sachet water in Nigeria is popularly called 'Pure Water'. The demand for safe drinking water in Nigeria cannot be overemphasized considering the inability of the government to provide adequate pipe borne water to the populace. However, safe drinking water is very scarce, the ever increasing demand of readily available water has led to the concept of pure water. Is this sachet water really safe for drinking? This study was carried out to examine the microbiological quality of sachet drinking water sold in Bayelsa and Rivers States, Niger Delta regions of Nigeria. Different brands of sachets water sold and consumed in Rivers and Bayelsa states (Niger Delta regions of Nigeria) were studied for the presence of Salmonella species and other enteric microorganisms between January and October 2012. Thirty five (35) sachets water from seven (7) different brands were serially diluted and cultured on selective media Mac Conkey, *Salmonella Shigella* and Eosin Methylene Blue agar and Nutrient agar for stock culture, while Muller Hinton agar was used for sensitivity test. Pure isolates suspension was standardized with 0.5 Mc Farland turbidity standard and were subjected to antibiotics susceptibility test using Agar Diffusion method and Biomark antibiotic disc. Bottle water was also tested as control. Various bacteria were isolated from all the test sachets water except the bottle water. The microbial counts ranged from  $1.9 \times 10^2$  Cfu/ml to  $6.3 \times 10^5$  Cfu/ml. Bacteria isolates include *Salmonella* sp. 16 strains, *Streptococcus* sp. 12 strains, *Klebsiella* sp. 6 strains, *Escherichia coli* 2 strains, *Bacillus* sp. 7 strains, *Enterococcus* sp. 4, and *Enterobacter* sp. 2. All the isolates were highly susceptible to Fluoroquinolones class of antibiotics, Ofloxacin (98.1%) Pefloxacin (99.2%), Levofloxacin (95.5%), Ciprofloxacin (99.5%), Streptomycin (94.4%) and Aminoglycosides Gentamycin (94.4%); moderately susceptible to Penicillin class, Amoxicillin (55.2%), Ampiclox (54.2%), Rifampicin (65.7%) and Macrolides, Erythromycin (64.1%) and highly resistant to Nalidixic Acid, Ampicillin and Ceporex. Statistical analysis showed no significant difference in the efficacy of the antibiotics susceptibility at ( $P < 0.05$ ). These findings showed that the quality of test sachets water does not meet the World Health Organization Standard and should be discouraged from drinking. The drug pattern in this study may serve as a guide in patient care.

**KEYWORDS:** Sachet water, Antimicrobial susceptibility, Salmonella species, Bacteria.

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## INTRODUCTION

Water is one of the most essential commodities for the survival of all lives. It is abundant in nature and occupies about 70% of the earth's crust (Botkin and Keller, 1998; Drinking Water Research Foundation, 2004). The demand for safe drinking water in Nigeria cannot be over emphasized considering the inability of the government to provide adequate pipe borne water to the populace. Well packaged water in bottle or food grade polyethylene sachets designed for stands is ready alternative for the growing population of over 140,000,000 people, however safe drinking water is very scarce, the ever increasing demand of readily available water has led to the concept of sachet pure water. It is a general perception in Nigeria that sachet water is safe for consumption and is popularly known as 'pure water' normally sold at the rate of five or ten naira per sachet. Continue increase in the sale and indiscriminate consumption of package drinking water in Nigeria is of Public Health significance. Most of the sachet water brands fall below World Health Organization (WHO) drinking water standards and are therefore of doubtful quality (Dibua, Esimone and Ndianefo, 2007). Need to be intensified in the monitoring of activities in this rapidly industry with a view to raising standards sachet water like any other food product must be processed and packaged under aseptic conditions free from every possible source of contamination, the portability of this water is found uncertain being collected from almost every available water source ranging from rain water to tanker- borne water most of which are rusty and unwashed (Dibua, Esimone and Ndianefo, 2007). Adherence to production and analytical standards are doubtful as most of the factories are observed to lack the appropriate technology for achieving these standards (Warburton and Austin, 1997). The quality monitoring of sachet water in Nigeria have been documented (Adekunle *et al.*, 2004; Onifade *et al.*, 2008; Dada, 2009; Gadner, 2004). However there is little information in scientific literatures on the quality of many brands of bottled and sachet water produced and marketed by local and multinational companies. Water borne diseases continue to be one of the major health problems especially in developing Nations like Nigeria. The high prevalence of diseases such as diarrhea, typhoid fever, cholera and bacillary dysentery among the populace has been traced to the consumption of contaminated water. (Adekunle *et al.*, 2007; Ibrahim *et al.*, 2000; Johnson, *et al.*, 2003). Public Health is the science and practice of protecting and improving the health of a community, as by preventive medicine, health education, control of communicable diseases, and application of sanitary measure and monitoring of environmental hazards (The America heritage dictionary). The focus of public health intervention is to improve health and quality of life through the prevention and treatment of disease and other physical and mental health condition through surveillance of cases and promotion of healthy behaviours. Promotion of hand washing is an example of common public health measures (Ogundipe, 2008). Public health plays important role in disease prevention efforts in both the developing world and in developed countries, through local health systems and non – governmental organizations. The World Health Organization (WHO) is the International agency that co- ordinates and acts on global public health issues. Most countries have their own government public health agencies, sometimes known as Ministries of Health to respond to domestic health issues. A reliable supply of clean wholesome of water is highly essential in a bid to promoting healthy living amongst the inhabitants of any defined geological regions (Mustapha and Adams, 1991). The standard industrialized world model for delivery of safe drinking water and sanitation technology is however not affordable in much of the developing world (Gadgil and Derby, 2003). There are now several brands of these type of packaged water marketed in Nigeria and other developing nation (Ogan, 1992; Kassanga, 2007). Water in sachets is readily available and the price is affordable but there are concern about its purity, integrity of the hygiene environment and the condition where the majority of sachet water is produced has also been questioned (Dada, 2009). It should however not be automatically assumed that packaged water in sachets is generally safe. Although the technologies used in these water industries present barriers that prevent pathogen presence in the final product. The quality of the packaged water is compromised significantly as it moves from the manufacturer to the consumer regulatory activities that promotes core hygiene values ( e.g hand washing, general cleanliness of storage environment and vendor container) and a proper handling culture could produce the desired improvements rather than a tenacious focus on end product monitoring which does not always give a complete picture in terms of microbiological risk assessment. This study aims to evaluate the microbiological quality of different brands of sachets water produced, marketed and consumed in Bayelsa and Rivers states, Nigeria with emphasis on Salmonella and other Enteric bacteria.



## MATERIALS AND METHODS

### Description of Study Area

Seven (7) different brands of sachet water and a brand of bottle water (as a control) were studied for their microbiological analysis. These water samples were collected from different areas in Rivers and Bayelsa states both in Niger Delta region. The areas include Oil Mill located in along East – West road in Port Harcourt (PH) and faces Eleme junction in the North, and Rumukrushu in the South. Rumuokoro is an area located along East – West road in PH, Mile 1 and Diobu are located in the heart of Port Harcourt. Mbiama is in Rivers State facing Ahoada, Bayelsa and Delta State in the North, East and South respectively. Yenagoa is an area located in the heart of Bayelsa State, Okolobiri and Efeka are in Bayelsa State but the latter is adjacent. The names of the sample companies and their locations are:

COMPANY	LOCATION	CITY	STATE
1 Skytop water	Oil Mill	Port Harcourt	Rivers State
2 Sonkists water	Rumuokoro	Port Harcourt	Rivers State
3 Brino water	Diobu	Port Harcourt	Rivers State
4 Ibis water	Mile 1	Port Harcourt	Rivers State
5 AFT water	Mbiama	Mbiama	Bayelsa State
6 P. A ,,	Okolobiri	Okolobiri	Bayelsa State
7 OHIS water	Efeka ama	Amassoma	Bayelsa State
8 EVA bottle water	Yenagoa	Yenagoa	Bayelsa State

### Sample Collection

Five (5) sachets each of water samples were collected from Skytop water (Oil Mill) Brino (Diobu), Ibis (Mile 1), AFT (Mbiama), Sonkist (Rumuokoro), PA (Okolobiri), Ohis (Efeka ama) and two bottles of Eva water (Yenagoa) all in Rivers and Bayelsa States.

### Research Areas

This research covered Oil Mill, Rumuokoro, Diobu, Mile 1, Mbiama in Rivers State and Yenagoa, Okolobiri and Amassoma in Bayelsa State.

### Materials and Media used

Sterile pair of scissors, Methylated spirit (Moko), sterile universal bottles, syringes (2ml), Cotton wool (Dr. Whiter), Petri dishes, Wire loop, autoclave, dryer, Sachet water, Bottle water, Hot air oven, Foil paper, bijou bottles, Water bath, Beakers, plasma, Incubator, colony counter.

### Media

Mac Conkey agar, Nutrient agar, Simmon Citrate agar, Nutrient broth, Eosin Methylene blue agar.

### Methods

#### Microbiological examination of the samples

Thirty five (35) brands of sachet water and two (2) bottle water were collected from different locations in Rivers and Bayelsa States for this study. The surface of the sachets water was swabbed with cotton wool soaked in methylated spirit and was used to swab the surface of the sterile scissors in order to prevent contamination. The swabbed surface was then cut open with a sterile pair of scissors and the different brands of sachet and bottle water were dispensed into sterile universal bottles. A differential medium Mac Conkey was used , it was prepared according to the manufacturer's instruction. 0.1 ml and 1.0 ml of serially diluted to  $10^3$  cfu/ml different water samples were inoculated into the medium in triplicate aseptically using a sterile syringe and incubated at  $37^{\circ}\text{C}$  for 24hrs. The colonies observed were counted using colony counter. Different colonies were sub cultured individually to obtain pure isolates which were then stored in a prepared stock culture bottles containing nutrient agar for further identification and characterization.

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### Identification and Characterization of the Bacteria Isolates

The water samples were inoculated on the appropriate selective media with a sterile wire loop. The media include *Salmonella- Shigella*, Eosin Methylene blue, Mac Conkey, Nutrient agar for stock culture and Muller Hinton agar for sensitivity test. The cultures were incubated at 37<sup>0</sup> C in an aerobic atmosphere. Pure cultures were used for the characterization of heterotrophic bacteria present in the water sample. The characterization was based on the criteria contained in CRC Handbook of Microbiology Bergeys manual of Determinative Bacteriology . Official Method of Analysis and Laboratory Methods in Food and Dairy Microbiology . The isolates were distinguished on the basis of (a) morphology and staining reactions (b) cultural characteristics and biochemical reactions.

Analysis of Variance (ANOVA) was done with the means values obtained from the zone of inhibition of susceptible antibiotics.

### RESULTS

Figure 1 depicted the graph of Antibiotics Susceptibility patterns of different microbial isolates from study sachet water samples. The results obtained after the inoculation and incubation of the test samples showed that different brands of sachet water were contaminated with various organisms. The microbial load before and after the dilution were determined using spread method, the microbial load ranged from 186 - 400 Cfu/ml and 1.7 – 6.3 (x10<sup>3</sup>) Cfu/ml (Colony Forming Unit/ml) respectively and there was no growth in the test bottle water that serve as a control experiment. The test water brands include Brino, Sonkist, Skytop, Ibiso, AFT, OHIS, PA and EVA bottle as control. The organisms isolated include *Streptococcus* sp, *Salmonella* sp 1, 2 and 3, *Escherichia coli* and *Enterobacter* sp, *Salmonella* sp1, 2 and 3, *Enterococcus* sp, *Streptococcus* sp 1,2,3,and 4; *Enterococcus* sp 1 and 2; *Bacillus* sp 1 and 2; *Enterobacter* sp. 1 and 2; *Salmonella* sp 1, 2 and 3; *Streptococcus* sp; *Salmonella* sp1,2 and 3, *Bacillus* sp 1, 2 and 3 and *Escherichia coli* respectively. *Salmonella* strains isolated from the test brands were 16 and predominant over other microbial isolates from these test water samples. The antibiotics tested include Ciprofloxacin, Norfloxacin, Gentamycin, Amoxycilin, Ampicillin, Rifampicin, Erythromycin, Levofloxacin, Streptomycin, Chloramphenicol, Pefloxacin, Tarivid, Ampicillin, Nalidixic Acid, Augumenyin, Ceporex and Septrin.

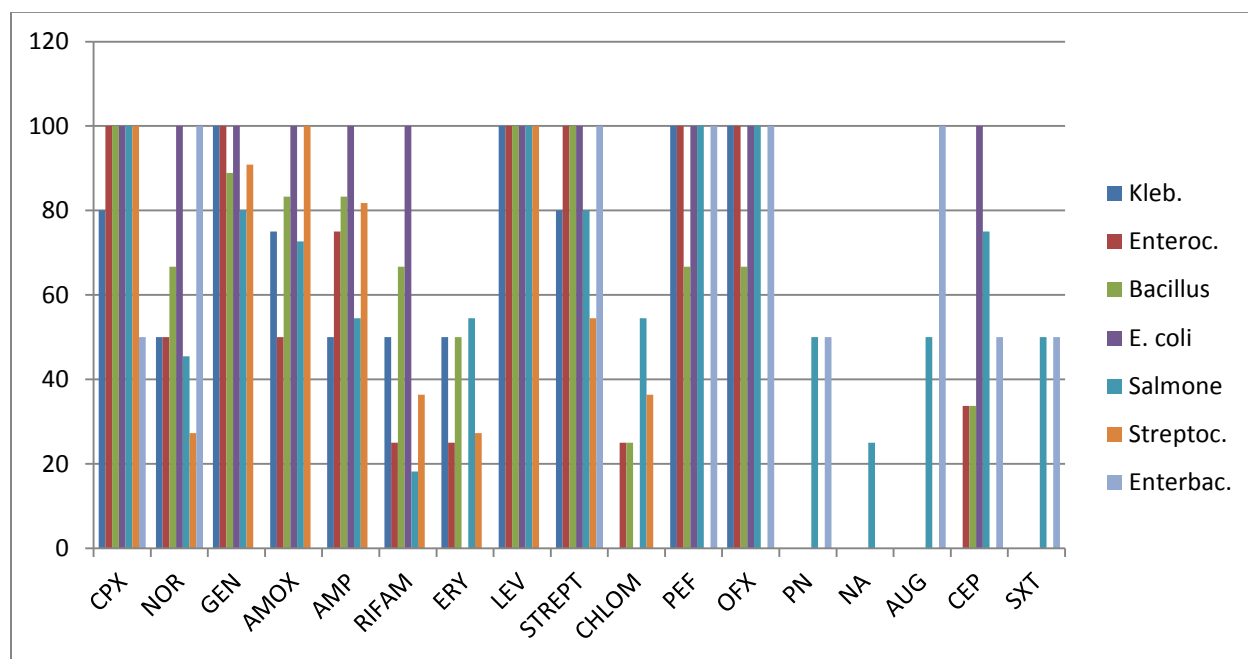


Figure 1.0 showing Antibiotic Susceptibility patterns of different Microbial Isolates from study Satchet Water.hh

### DISCUSSION

*Salmonella* sp., *Bacillus* sp., *Streptococcus* sp., and *Enterobacter* sp., were highly susceptible to Ciprofloxacin (100%), while *Klebsiella* sp *Escherichia coli* *Enterococcus* sp., *Bacillus* sp., *Streptococcus* sp., and *Salmonella* sp were highly susceptible to Levofloxacin (100%). All the isolates were highly susceptible to Fluoroquinolones class of antibiotics, Ofloxacin (98.1%) Pefloxacin (99.2%), Levofloxacin (95.5%), Ciprofloxacin (99.5%), Streptomycin (94.4%) and Aminoglycosides Gentamycin (94.4%); moderately susceptible to Penicillin class, Amoxicillin (55.2%), Ampiclox (54.2%), Rifampicin (65.7%) and Macrolides, Erytromycin (64.1%) and highly resistant to Nalidixic Acid, Ampicillin and Ceporex. Access to good quality of water has a challenge to humans in developing countries such as Nigeria due to pollution of available water. This phenomenon is leading to the loss of children below the age of five (5) on an hourly basis with the rate at which sachet water producing companies are emerging, there is need to access the quality of the water being produced for consumption. The sachet water supposed to be safe, hygiene affordable and an instant source of water for consumers. During this research , seven (7) brands of different sachet water and a brand of bottle water were analyzed for their microbiological standard and from the results obtained, it was found that these samples contained more than one coliform and other pathogenic organisms which do not conform to WHO standard for drinking water and states that portable water for consumption must be free of microbial indicators of faecal contamination and coliform count per 100 ml of drinking water must be zero (World Health Organization, 2002). The presence of coliforms indicates an ‘alarm’ system this is because coliforms are indicator microorganism whose presence in water is evidence that water is feacally contaminated and the organisms isolated from the test samples (sachets water) include *Salmonella*, *Echerichia coli*, *Klebsiella* species, *Enterococcus faecalis*, *Salmonella* species, *Enterobacter* species and *Streptococcus* species, ingestion of water containing these organisms poses health hazard to the consumers and may lead to deleterious effects. *Salmonella* is a gram negative bacterium, and they cause illness like Typhoid fever, Paratyphoid and food borne illness. Many are due to ingestion of contaminated food and water. Other enteric bacteria are found in the digestive tract of humans and animals and are of different types. Water is an important vehicle of various microorganism notably bacteria and viruses, the primary source of these pathogens are sewage the environmental bodies receives a significant amount of treated, partially treated, or untreated sewage which severely depletes the water quality hence if the treatment is insufficient or if the distribution is inadequate drinking water may contain sufficient pathogens to cause illness (O’ Connor, 2002).

### CONCLUSION

In conclusion, this study has X- rayed the level of contamination the study sachet water samples have, proper hygiene should be practiced in our production sites in order to have safe drinking water for the populace, since many people cannot afford bottle water in the country. Federal Government delegates should be sent out for effective monitoring of these personnel. Any water production industry that violates the standard should be closed down and bound from production.

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