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Presence of Antibiotic Resistant Bacteria in Sachet Water Produced and Sold in the Eastern Nigeria

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Abstract: Sachet water samples produced locally were examined for quality and antibiotic resistant bacteria. The total viable counts and types of bacteria in the water samples were determined using standard bacteriological techniques. The number of organisms obtained from the water samples ranged from 1.2×10^1 to 3.0×10^2 cfu/100 mL. Bacterial isolates resistant to ampicillin were 80.4% while 77.2% were resistant to tetracycline and 23.7% were resistant to ofloxacillin. The presence of these antibiotic resistant organisms in drinking water is of public health importance and may be responsible for prolonged treatment of water borne diseases. Therefore proper diagnosis and appropriate administration of drugs will help in controlling the increase of antibiotic resistant bacteria. However, proper treatment of water before consumption is recommended.

Key words: Sachet water, microbial quality, antibiotic resistant bacteria

INTRODUCTION

Sachet or packaged water is any water that is in sealed plastic and distributed or offered for sale and is intended for human consumption (Food and Drug Administration, 1995). Sachet water is widely available in both developed and developing countries. Consumers have many various reasons for purchasing sachet water which include tastes, conveniences, price and safety. Packaged water comes in various sizes ranging from 60 mL to 2 L. Depending on the climate, physical activities and culture, the drinking water needs for individual vary but for high consumers it is estimated to be about two litres per day for a 60 kg person and one litre per day for a 10 kg child as stated by Health Canada (1981).

Potential health problems may exist due to the microbial content of the sachet water since water is one of the vehicles for the transmission of pathogenic organisms (Brock, 1991; Prescott *et al*., 2005). However, the type of organisms present depends on a number of factors such as the type of soil over which the water flows, contamination by animals, sewage and agricultural waste (Hunter, 1993). Water pollution is a major course of illness (Craun, 1988; Harrison, 1992; Nwachukwu, 2001). Microbial pathogens associated with water pollution include *Salmonella*, *Shigella*, *Vibrio*, *Campylobacter*, *Yersinia*, *Cryptosporidium* and *Giardia* species (WHO, 1996).

Bacteria with intrinsic resistance to antibiotics are found in nature. Some organisms may acquire additional resistant genes from bacteria introduced into water or soil. Antibiotic resistant gram-negative bacteria belonging to the following genera, *Acinetobacter*, *Alkaligenes citrobacter*, *Enterobacter*, *Pseudomonas* and *Serratia* isolated from rivers were reported by Ash (2002). The natural cause of antibiotic resistance is mutation or R-plasmid exchange between bacteria of the same species (Bell *et al*., 1980). The common use of antibiotic in poultry or agricultural practices to prevent diseases

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can contaminate surface and underground water (El-Zanfaly *et al*., 1988) from where they can be transferred to humans in drinking water thereby adding to the resistance problem. Also, the inappropriate use of antibiotic in preventing or treating human infections is believed to be the common cause of bacterial resistance. When these antibiotic resistant bacteria are introduced into water through faecal contamination, its emerging diseases will hardly respond to treatment. It is common that bacteria that develop resistance to one antibiotic may also have the ability to develop resistance to another antibiotic which is usually referred to as multiple-antibiotic resistance (Walter and Vennes, 1985).

Therefore in view of the large number of possible hazards associated with drinking water the objective of this study is to determine the quality and the antibiotic resistant bacteria in sachet water.

MATERIALS AND METHODS

Sources of Samples

Sixty sachet water samples were randomly purchased from different sale points in Aba and Owerri, Nigeria. The names of the sachet water included Mevok, Ragolis, Divine, Pasun, Agad, Vince, Victory, Ben, Imobest and Goodness.

Enumeration of Bacteria in Water Samples

Employing the filtration techniques (APHA, 1992), 100 mL of each water sample (based on the producer's trade name) was filtered through membrane (0.4 μ pore size). There after the membrane filter was placed aseptically (using flame sterilized forceps) on nutrient agar plates (for the enumeration of heterotrophic bacteria), Eosin methylene blue agar (for total coliforms) and mannitol salt agar plates (for selective growth of *Staphylococcus*). The experiment was carried out two times for each water samples. All the inoculated plates were incubated at 35±2°C for 48 h and then the colonies were counted and recorded.

The colonies observed on different media (Nutrient, Eosin methylene blue and mannitol salt agar plates) were sub cultured to obtain pure cultures. Isolates were then identified by carrying out some tests which included gram staining, coagulase, catalase, oxidase, indole production, citrate utilization methyl red and voges proskauer and sugar fermentation (Harrigan and McCance, 1976).

Antibiotic Susceptibility Testing

Antibiotic susceptibility was determined by the agar diffusion technique (Baker and Breach, 1980) using 8 antibiotic disks (Biotec Lab. United Kingdom) corresponding to the drugs most commonly used in the treatment of human and animal infections caused by bacteria (ampicillin, tetracycline, ofloxacillin, cotrimoxazole, ampiclox, gentamycin, amoxacillin and augmentin). After 24 h of incubation at 37°C, bacteria were classified as sensitive or resistant based on the zone of inhibition.

RESULTS

A higher value of total viable counts was 200 cfu mL^{-1} on nutrient agar while on Eosin methylene blue agar plate it was 100 cfu mL^{-1} and on the mannitol salt agar plate it was 8 cfu mL^{-1} (Table 1). The highest number of organisms (on all the media) was 300 cfu mL^{-1} (3.0×10^{2} cfu mL^{-1}) in Ben sachet water samples and the lowest was 12 cfu mL^{-1} (1.2×10^{1} cfu mL^{-1}) in mevok sachet water samples. A greater percentage (80.4%) of bacteria was resistant to ampicillin and 77.2% was resistant to tetracycline while 23.7% of bacteria were resistant to ofloxacin (Table 2).

Table 1: Total Viable Counts (TVC) in water samples on different media

Water samples	No. of TVC on NA	No. of TVC on EMBA	No. of TVC on MSA	Total No. of organisms (cfu mL^{-1})
Mevok	10	2	0	1.20×10^{1}
Ragolis	12	3	0	1.50×10^{1}
Divine	20	7	0	2.70×10^{1}
Pasun	100	35	0	1.35×10^{2}
Agad	123	2	8	1.33×10^{2}
Vince	135	6	1	1.42×10^{2}
Victory	25	5	0	3.00×10^{1}
Ben	200	100	0	3.00×10^{2}
Imobest	100	10	2	1.12×10^{2}
Goodness	15	13	0	2.80×10^{1}

NA: Nutrient Agar; EMBA: Eosin Methylene Blue Agar; MSA: Mannitol Salt Agar

Table 2: Percentages of bacteria resistant to antibiotic

Antibiotic (μg/disk)	Resistant (%)
Ampicillin (10)	80.4
Tetracycline (30)	77.2
Ofloxacin (10)	23.7
Cotrimoxazole (30)	57.5
Ampiclox (30)	64.4
Gentamycin (10)	33.9
Amoxacillin (30)	47.3
Augmentin (30)	39.0

DISCUSSION

Sachet or packaged water samples were analyzed to determine the microbial quality and the antibiotic resistance patterns among the bacterial isolates with a view to create awareness about the public health implications of drinking such water. The result of the total viable counts showed colonies of up to 100 to 200 in some water samples. This indicated possible contaminations of the water samples. World Health Organization (1996) guideline values for bacteriological quality for all water intends for drinking stated that *Escherichia coli* or total coli form bacteria must not be detectable in any 100 mL samples. Therefore, by the WHO standard some of the sachet water were not safe for human consumption. The total viable counts on Eosin methylene blue agar plates for coliform bacteria and the various values obtained for each water samples signified possible faecal contamination. This may be that some of the packaged water were prepared from shallow and contaminated boreholes. Other bacteria such as *Staphylococcus* isolated from the water samples may have entered the water during packaging or handling (Hunter, 1993) since the organisms are normal flora of the human skin. However, the presence of the organisms in drinking water is of public health importance because it is usually responsible for staphylococcal food poisoning (Hobbs and Robert, 1993; Frazier and Westhoff, 1995).

The result of the antibiotic susceptibility testing showed various percentages of antibiotic resistance among the bacterial isolates from packaged water samples. The presence of antibiotic resistant bacteria in drinking water is of health significance because of the danger in promoting multiple antibiotic resistant organisms in humans. The prevalence of drug resistant organisms has imposed a great challenge to clinicians. And this study has provided a guide to the possible cause of antibiotic resistance of bacteria which could be promoted by drinking especially untreated packaged water. The consumption of water containing these antibiotic resistant organisms may prolong the treatment of water-borne diseases.

Higher percentages of bacterial isolates were resistant to ampicillin and tetracycline but low percentage were resistant to ofloxacin. Similarly, sixty three Gram negative bacteria isolated from rural untreated drinking stream water supply demonstrated high resistance to ampicillin (Nwachukwu and Otokunefor, 2003). In another study, 87% of coliforms in ground waters supplies were resistant to at least one antibiotic with resistance most commonly directed towards novobiocin, cephalothin and ampicillin (McKeon *et al*., 1995). Surprisely, these bacterial isolates were resistant to commonly used antibiotic such as ampicillin, cotrimoxazole, ampiclox, tetracycline and amoxicillin. This implies that the treatment of water borne diseases with these antibiotics may be inappropriate and will require new antibiotics which are not commonly used. Antibiotic resistant bacteria are a cause for concern because of possible colonization of the gastrointestinal tract and conjugal transfer of antibiotic resistance to the normal flora leading to more multiple antibiotic resistant organisms (McKeon *et al*., 1995).

It has been observed that many people are involved in the production and selling of sachet or packaged water as a source of income. Therefore, health authorities should ensure that producers comply with the government regulations since some of these packaged water may have been prepared under unhygienic conditions. The water main for human consumption most be treated and the necessary biochemical and microbiological tests should be carried out so as to protect the public from water-borne disease outbreak.

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